MAJOR ARTICLE

Safety and Immunogenicity of 2009 Pandemic H1N1 Influenza Vaccination in Perinatally HIV-1–Infected Children, Adolescents, and Young Adults

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Background. The safety and immunogenicity of high-dose pandemic H1N1 (pH1N1) vaccination in perinatally human immunodeficiency virus type 1 (HIV-1)–infected children, adolescents, and young adults are unknown.

Methods. Two 30-μg doses of 2009 Novartis pH1N1 monovalent vaccine (Fluvirin) were administered 21–28 days apart to perinatally HIV-1–infected children, adolescents, and young adults. Antibodies were measured by hemagglutination inhibition (HAI) assay at baseline, 21–28 days after first vaccination, 7–13 days after the second vaccination, and 7 months after the first vaccination.

Results. Among the 155 participants, 54 were aged 4–8 years, 51 were aged 9–17 years, and 50 were aged 18–24 years. After 2 doses of Fluvirin, seroresponse (≥4-fold rise in HAI titers) was demonstrated in 79.6%, 84.8%, and 83% of participants in the aforementioned age groups, respectively, and seroprotection (HAI titers ≥40) was shown in 79.6%, 82.6%, and 85.1%, respectively. Of those lacking seroresponse (n = 43) or seroprotection (n = 37) after the first vaccination, 46.5% and 40.5% achieved seroresponse or seroprotection, respectively, after the second vaccination. Among participants who lacked seroprotection at entry, a "complete response" (both seroresponse and seroprotection) after first vaccination was associated with higher baseline $\log_{10}$ HAI titer and non-Hispanic ethnicity. No serious vaccine-related events occurred.

Conclusion. Two doses of double-strength pH1N1 vaccine are safe and immunogenic and may provide improved protection against influenza in perinatally HIV-1–infected children and youth.

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A novel swine-origin influenza A subtype H1N1 virus, designated 2009 H1N1 influenza A, was identified as the cause of pandemic febrile respiratory illnesses [1–4]. Although individuals of all ages were affected, the greatest increase in severe morbidity and mortality occurred in young children, pregnant women, and the morbidly obese [5]. In human immunodeficiency virus type 1 (HIV-1)–infected patients, influenza infection is more severe than that typical of age-matched uninfected people [6, 7]. HIV-1–nfected

patients also may shed virus for greater periods of time, prolonging the need for isolation in the clinic or hospital [8]. Because of the increased severity of this pandemic in children and young adults, knowledge of the safety and immunogenicity of the pandemic influenza A (pH1N1) 2009 monovalent vaccine in this population is critically important.

Antibody responses to seasonal trivalent influenza vaccine (TIV) are blunted in HIV-1–infected children and adults who are not receiving antiretroviral therapy (ART) [9–11] but improved in patients who do not have progressive HIV-1 disease and/or are receiving combination ART (cART) [12–16]. Still, the immunological response is poorer compared to that of HIV-uninfected cohorts [15, 16]. Studies evaluating the effect of antigen dosage on the immune responses to TIV performed over the past 35 years demonstrate dose-related increases in serum and mucosal antibody responses [17–25]. Higher vaccine dosages are also associated with the development of higher levels of serum antibodies that recognize antigenically distinct drift variants [23] and can overcome suboptimal responses in immunologically impaired vaccinees, such as elderly patients [22, 24, 25]. However, higher dosages of hemagglutinin are also associated with more frequent adverse events.

We hypothesized that 2 doses of influenza vaccine would be necessary to achieve protection in perinatally HIV-1–infected children and youth who had no prior exposure to pH1N1. In addition, because of blunted response to TIV in HIV-1–infected persons, we investigated a 30-μg dose of antigen, double the 15-μg dose proposed for healthy children.

METHODS

Perinatally HIV-1–infected children, adolescents, and young adults, aged 4–24 years, were recruited from International Maternal Pediatric and Adolescent Clinical Trials (IMPAACT) group units in the United States and Puerto Rico. Participants were either receiving stable ART for at least 90 days prior to entry or no ART within 90 days prior to entry. Participants were excluded for platelet count ≤50 000/μL or absolute neutrophil count ≤500/μL within 30 days prior to study entry; known allergy to vaccine components; history of severe reactions after influenza vaccination; known pH1N1 infection or vaccination; receipt of a live vaccine within the prior 4 weeks or inactivated vaccine in the prior 2 weeks; receipt of immunoglobulin or other blood products within the prior 3 months; immunosuppressive condition other than HIV infection; personal or family history of Guillain-Barré syndrome (GBS); or onset of neurological disorder characterized by loss of strength or reflexes within the prior 6 months. Prior to the second vaccination, the participants were required to meet the same inclusion and exclusion criteria. Female participants of childbearing potential were required to have a negative pregnancy test within 72 hours before each vaccination.

Vaccine Administration

Participants received two 30-μg doses of 2009 Novartis influenza A (H1N1) monovalent vaccine (Fluvirin) separated by 21–28 days. Each 30-μg vaccine dose was administered in the deltoid or anterolateral thigh muscle as two 0.5-mL (15-μg) injections. Participants had assessment of vaccine safety using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0 (http://rsc.tech-res.com/Document/safetyandpharmacovigilance/Table_for_Grading_Severity_of_Adult_Pediatric_Adverse_Events.pdf). Participants were observed for at least 30 minutes after each vaccination and were contacted by telephone or other methods for reactogenicity assessments and safety monitoring on day 2 (±1 day) and day 10 (±3 days) after first vaccination and then again on day 2 (±1 day) after second vaccination; they were also seen on day 10 (±3 days) after second vaccination. Report of any symptom compatible with GBS (eg, weakness of legs, tingling of hands and/or feet, or difficulty walking) required a clinic visit within 24 hours of onset.

Immunogenicity Assessments

Immunogenicity was assessed by specific hemagglutination inhibition (HAI) titers in serum collected at baseline (the study entry visit), 21–28 days after first vaccination, 7–13 days after second vaccination (if received), and 7 months after first vaccination. Timing of the assessment following second vaccination was selected to coincide with the expected peak of an anamnestic response. Henceforth, these time points will be called *baseline*, *after first vaccination*, *after second vaccination*, and *7 months after first vaccination*. The assay was adapted from previously described methods developed and validated for seasonal influenza viruses [12]. HAI titers ≥40 was defined as evidence of seroprotection [26]. Seroresponse was defined as having a ≥4-fold rise in HAI titers following vaccination as compared with baseline HAI. A *complete responder* was defined as a participant who achieved both seroprotection and seroresponse, regardless of baseline serology.

Statistical Analysis

The baseline characteristics and safety data for all participants enrolled in the study were summarized using descriptive measures. The HAI titers following the first vaccination were summarized for the eligible study participants who received at least 1 dose of vaccine; the titers following the second vaccination were summarized for the eligible study participants who received both doses of vaccine. Three study participants with pH1N1 infection during the study (after first vaccine) were excluded from all HAI analyses but not safety analysis. The serology analyses were stratified by 3 age groups: 4–8 years, 9–17 years, and 18–24 years.

HAI titers <10 were considered undetectable and were assigned a value of 5 for this analysis. The titers were

summarized using geometric means and 95% confidence intervals (CIs) as well as medians. A Sign test was used to determine, within each age group, whether the number of participants showing increased titers from baseline to follow-up was greater than the number showing decreases. Rates of seroprotection (HAI titers ≥40) were computed, as well as fold changes from the titer values at baseline. Differences in the seroprotection and seroresponse rates after vaccination among the age groups as well as the differences in rates between those with detectable antibody at baseline (HAI titers ≥10) and those without were assessed using Fisher exact test. Among the study participants without seroprotection at baseline, the exact McNemar test of agreement was used to compare the rate of seroprotection after the second vaccination to the rate after the first vaccination. The persistence of seroprotective levels 7 months after vaccination was assessed, and the rates of participants with seroprotection at present up to 7 months after vaccination were computed.

Univariate logistic regression analysis was used to assess the association of demographic characteristics (age, sex, ethnicity [Hispanic vs other], race [black vs other]), use of cART at study entry, viral load [<400 copies/mL or ≥400 copies/mL], TIV vaccination prior to study entry, CD4 count and percentage, CD8 count and percentage, CD19 count and percentage, and $\log_{10}$ HAI titer at baseline) with serologic response following first and second vaccination. Combination ART was defined as a regimen containing at least 3 ART drugs from at least 2 drug classes. Data from all age groups were combined. For these analyses, participants with baseline HAI titers ≥40 were omitted to avoid a mixture of primary and secondary response to the pH1N1 antigen. Multivariable logistic regression modeling with backward selection was used to evaluate the association of the above factors on immunologic response, including all factors with $P < .1$ in univariate models as candidate predictors; the final model retained only those covariates with $P < .05$. The analyses were performed using SAS software, version 9.2 (SAS Institute), and the graphs were produced using the R software package.

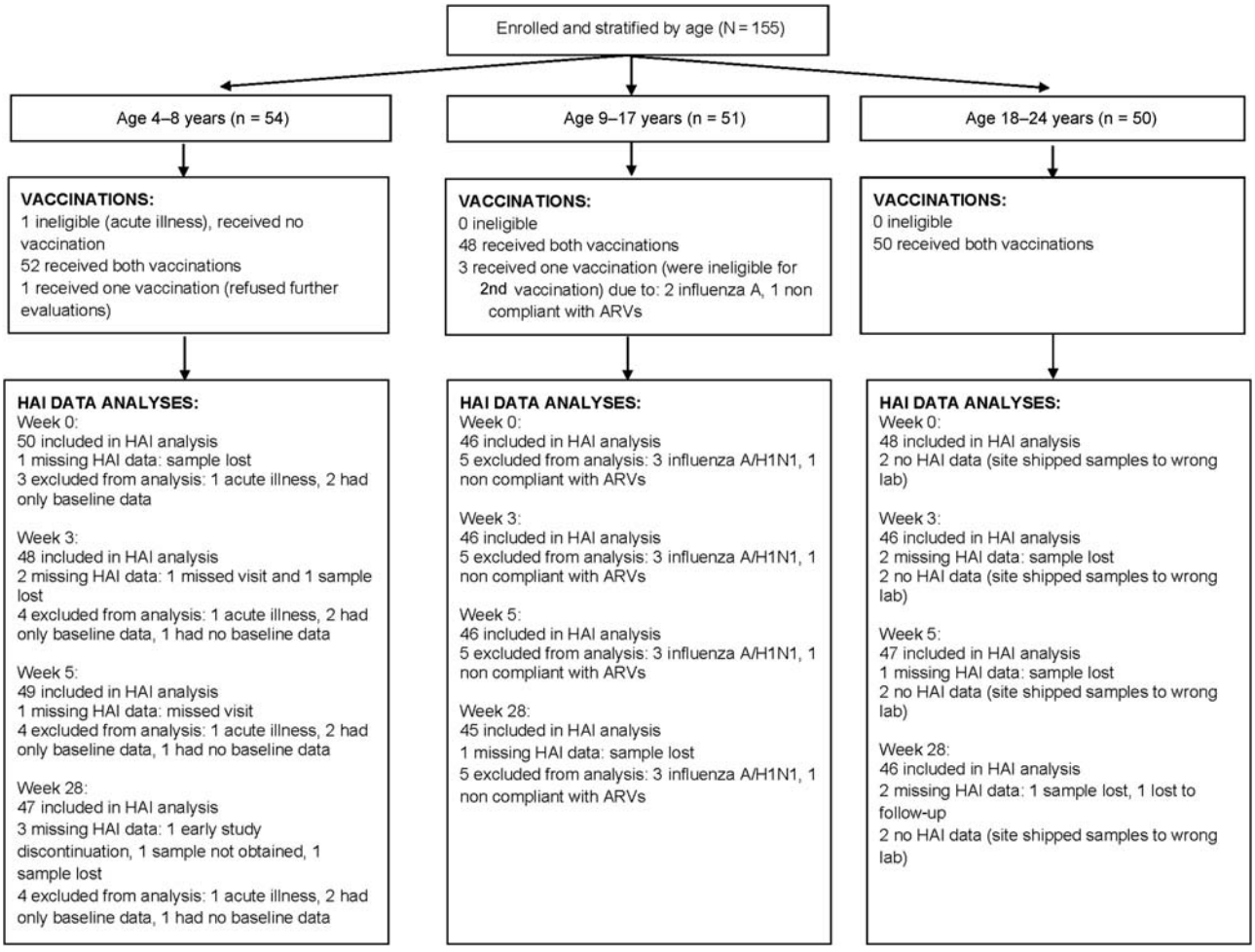

Figure 1. Patient flowchart after enrollment. Abbreviations: ARVs, antiretrovirals; HAI, hemagglutination inhibition titers.

RESULTS

We enrolled 155 children, adolescents, and young adults from 37 sites, between 14 October 2009 and 12 November 2009; 54 participants were aged 4–8 years; 51 were aged 9–17 years; and 50 were aged 18–24 years. Among the 155 participants, 150 (97%) received both vaccinations, 4 (2%) received 1 vaccination, and 1 received no vaccination (Figure 1). Reasons for not receiving the second vaccination were documented pH1N1 infection after first vaccination (n = 2), refusing further follow-up (n = 1), and ineligibility due to nonadherence to ARV medications (n = 1). The participant who received no vaccinations became ineligible due to an acute illness after enrollment but prior to vaccination. Demographic characteristics, CD4 percentage, and viral load are displayed in Table 1.

Safety

The vaccine was well tolerated; 12 grade 3 events were reported, including only 2 (both fever episodes) reported as possibly related to vaccine administration. Fever of 39.5°C was reported in 2 participants 3 and 7 days following first vaccination, respectively. The unrelated events included low neutrophil count (2), pH1N1 infection, tonsillitis, pharyngitis, sinusitis, dizziness, headache, neck pain, and nasal congestion (1 each). There were 10 grade 2 local and systemic events that were related to vaccine including injection site pain (3); tenderness (2); and itching, nausea, vomiting, headache, and leg pain (1 each). Seven of these events occurred in 3 participants 1–7 days following the first vaccination and 3 events occurred in 2 participants 1 day to 6 months following the second vaccination. Two participants had grade 2 herpes virus reactivation possibly related to vaccination, 1 with unilateral facial nerve palsy associated with an oral herpes simplex reactivation on day 4 after the second dose and 1 with dermatomal herpes zoster eruption 14 days after the first dose. There were no reported cases of GBS.

Influenza-like Illness

Seven study participants developed influenza-like illness during the course of the study. Six occurred 4–16 days following the first vaccination and 1 occurred 23 days after the second vaccination. Real-time reverse transcriptase polymerase chain reaction testing identified 3 as having influenza A: 2 confirmed pH1N1 influenza and 1 probable, based on local epidemiology. All influenza A infections occurred in the 9–17-year age group and were reported 6, 14, and 16 days following the first vaccination. Baseline HAI in these participants was 5, 10, and 160, respectively, and CD4 percentage was 16, 21 and 30, respectively.

Immunogenicity

HAI titers were available for 140 participants following the first vaccination and for 142 participants following the second vaccination (Tables 2 and 3 and Figure 2). The median, range, geometric mean titers, and 95% CI HAI titers are presented in Table 2.

Table 1. Study Participant Baseline Characteristics

	Age Group		
	4–8 y, n = 54 (35%)	9–17 y, n = 51 (33%)	18–24 y, n = 50 (32%)
Sex, male	54%	57%	42%
Race			
Black	65%	55%	64%
White	26%	39%	30%
Ethnicity			
Hispanic	33%	39%	24%
Age, y (median)	6	14	19
CD4 count, cells/μL, median (range)	1161 (308–1921)	642 (113–1488)	589 (57–1031)
CD4%, median (range)	37% (24–50)	33% (11–47)	30% (5–48)
Viral load, copies/mL, median (range)	48 (40–19K)	48 (40–80K)	75 (40–81K)
Baseline ART			
Combination ART[a]	50	45	42
Other[b]	1	3	4
None	3	3	4

Abbreviation: ART, antiretroviral therapy.

[a] Combination ART is defined as a regimen containing at least 3 antiretroviral drugs from at least 2 drug classes.

[b] Includes regimens with nucleoside reverse transcriptase inhibitor only, combination protease inhibitor and nonnucleoside reverse transcriptase inhibitor, and other combinations of antiretroviral agents.

Table 2. Summary of Hemagglutination Inhibition Titers

Age	Time Point	Median (Range)	No.	GMT (95% CI)	*P* Value[a]
All children	Baseline	10 (5–1280)	144	16 (13–19)	…
	After first vaccination	80 (5–1280)	140	85 (65–111)	<.0001
	After second vaccination	160 (5–1280)	142	127 (101–159)	<.0001
	7 mo after first vaccination	40 (5–640)	138	33 (27–40)	<.0001
4–8 y	Baseline	10 (5–1280)	50	16 (11–22)	…
	After first vaccination	60 (5–1280)	48	71 (44–116)	<.0001
	After second vaccination	160 (10–1280)	49	119 (78–180)	<.0001
	7 mo after first vaccination	20 (5–640)	47	27 (19–38)	.0002
9–17 y	Baseline	10 (5–80)	46	13 (10–16)	…
	After first vaccination	160 (5–1280)	46	94 (60–150)	<.0001
	After second vaccination	160 (5–1280)	46	134 (90–198)	<.0001
	7 mo after first vaccination	40 (5–160)	45	44 (34–57)	<.0001
18–24 y	Baseline	20 (5–320)	48	21 (14–30)	…
	After first vaccination	120 (5–1280)	46	92 (57–148)	<.0001
	After second vaccination	160 (10–1280)	47	128 (87–190)	<.0001
	7 mo after first vaccination	40 (5–320)	46	30 (21–45)	.006

Abbreviations: CI, confidence interval; GMT, geometric mean titer.

[a] *P* value from Sign test to test whether the no. of participants showing increased titers from baseline to follow-up was greater than the no. showing decreases.

Seroresponse

Overall, seroresponse occurred in 68.6% of participants following the first vaccination and increased to 82.4% after the second vaccination. There was a significantly higher rate of seroresponse after the second vaccination as compared to the first ($P < .0001$). Of those who lacked seroresponse after the first vaccination (n = 43), 46.5% achieved it after the second vaccination. Only 1 (1%) of those who had seroresponse after the first vaccination lost it after the second vaccination. Seroresponse rates were not related to age ($P = .20$ after first and $P = .82$ after second vaccination). Seroresponse was greater at all time points among participants with baseline HAI titers ≥10 (Table 4).

Seroprotection

Seroprotection was demonstrated in 32.6% at baseline, increasing to 72.1% after the first vaccination and to 82.4% after the second. As with seroresponse, seroprotection was not related to age ($P = .36$ after first vaccination and $P = .79$ after second vaccination). In participants who were evaluated after both vaccinations and who were without seroprotection at baseline (n = 93), 59.3% and 73.6% achieved it after the first and second vaccinations, respectively. There was a significantly higher rate of seroprotection after the second vaccination as compared to the first ($P = .002$). Of those who lacked seroprotection after the first vaccination (n = 37), 40.5% achieved it after the second vaccination. Only 2 (3.7%) of those who had

Table 3. Hemagglutination Inhibition Assay Findings Among Participants

	Seroresponse (≥4-Fold Rise in HAI)		Seroprotection (HAI Titers ≥40)			
Age	After First Vaccination	After Second Vaccination	Baseline	After First Vaccination	After Second Vaccination	7 mo After First Vaccination
All participants						
4–8 y	34/48 (70.8%)	39/49 (79.6%)	13/50 (26%)	31/48 (64.6%)	39/49 (79.6%)	19/47 (40.4%)
9–17 y	35/46 (76.1%)	39/46 (84.8%)	13/46 (28.3%)	35/46 (76.1%)	38/46 (82.6%)	36/45 (80.0%)
18–24 y	27/46 (58.7%)	39/47 (83%)	21/48 (43.8%)	35/46 (76.1%)	40/47 (85.1%)	24/46 (52.2%)
All	96/140 (68.6%)	117/142 (82.4%)	47/144 (32.6%)	101/140 (72.1%)	117/142 (82.4%)	79/138 (57.2%)
Participants with baseline HAI titers <40						
4–8 y	22/35 (62.9%)	27/36 (75%)	0/37 (0%)	18/35 (51.4%)	26/36 (72%)	8/35 (22.9%)
9–17 y	23/33 (69.7%)	27/33 (81.8%)	0/33 (0%)	22/33 (66.7%)	25/33 (75.8%)	23/32 (71.9%)
18–24 y	14/25 (56%)	23/26 (88.5%)	0/27 (0%)	14/25 (56%)	19/26 (73%)	7/25 (28%)
All	59/93 (63.4%)	77/95 (81.1%)	0/97 (0%)	54/93 (58.1%)	70/97 (73.7%)	38/92 (41.3%)

Abbreviation: HAI, hemagglutination inhibition.

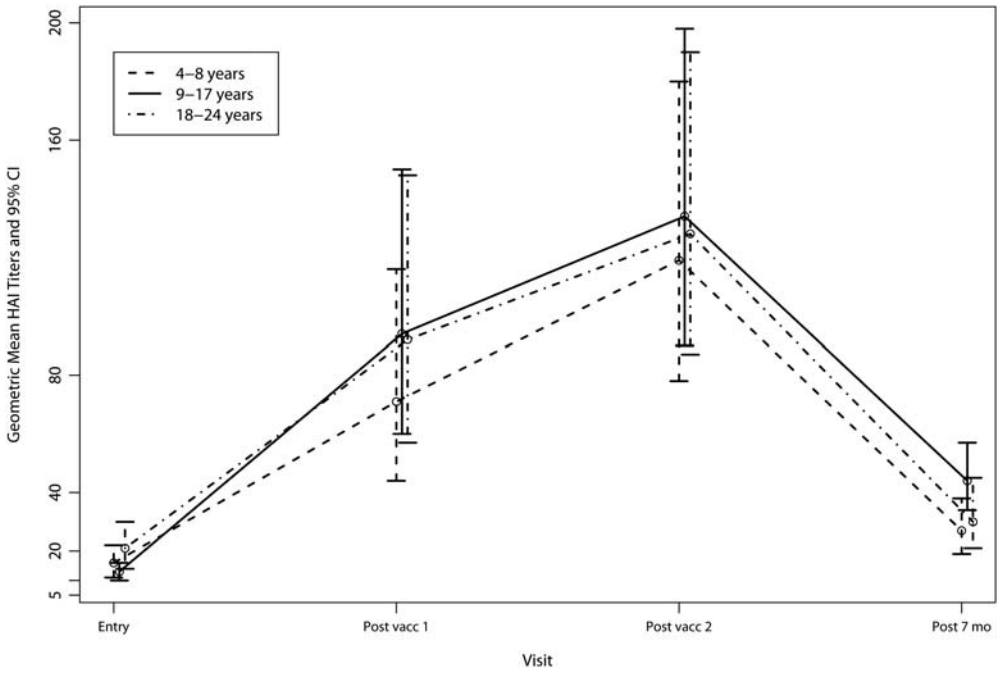

Figure 2. Geometric mean and 95% confidence interval (CI) hemagglutination inhibition (HAI) titers among vaccine recipients by age group.

seroprotection after the first vaccination lost it after the second vaccination. Seroprotection was greater following first vaccination and 7 months after first vaccination among participants with baseline HAI titers ≥10 (Table 4).

Complete Response

Complete response (both seroresponse and seroprotection) was achieved in 61.7%, 73.9%, and 58.7% of participants in the 4–8-, 9–17-, and 18–24-year age groups after first vaccination, respectively. Among those who were not complete responders after 1 vaccination, 9 of 18, 4 of 12, and 6 of 18 were complete responders after the second vaccination in the 4–8-, 9–17-, and 18–24-year age groups, respectively. Only 1 of 29, 1 of 34, and 0 of 27 who were complete responders after the first vaccination were not complete responders after the second vaccination in the 4–8-, 9–17-, and 18–24-year age groups, respectively ($P = .02$, .38, and .03, respectively). Overall, 39.6% (19 of 48) of those who were not complete responders after first vaccination became complete responders after the second vaccination, whereas 2.2% (2 of 90) of those who were complete responders after first vaccination were no longer complete responders after second vaccination

Table 4. Proportion of Participants With Seroresponse, Seroprotection, and Complete Response by Baseline Hemagglutination Inhibition (HAI) Titers

	Baseline HAI Titers <10	Baseline HAI Titers ≥10	*P* Value
Seroresponse			
After first vaccination	38.1% (16/42)	86.7% (85/98)	<.0001
After second vaccination	63.6% (28/44)	90.8% (89/98)	.0002
7 mo after first vaccination	27.9% (12/43)	70.5% (67/95)	<.0001
Seroprotection			
After first vaccination	52.4% (22/42)	75.5% (74/98)	.01
After second vaccination	79.5% (35/44)	83.7% (82/98)	.63
7 mo after first vaccination	51.2% (22/43)	25.3% (24/95)	.004
Complete response			
After first vaccination	38.1% (16/42)	76.3% (74/97)	<.0001
After second vaccination	63.6% (28/44)	84.5% (82/97)	.008
7 mo after first vaccination	27.9% (12/43)	25.5% (24/94)	.84

Abbreviation: HAI, hemagglutination inhibition.

Table 5. Baseline Factors and Relationship to Pandemic H1N1 Vaccine Response (Seroresponse and Seroprotection) After First and Second Vaccinations

	After First Vaccination[a]				After Second Vaccination[a]			
Characteristic	OR (95% CI)	*P* Value[b]	AOR (95% CI)	*P* Value[c]	OR (95% CI)	*P* Value[b]	AOR (95% CI)	*P* Value[c]
Male sex	1.0 (.4–2.3)	.98			0.6 (.2–1.4)	.23		
Hispanic ethnicity	0.4 (.2–1.0)	.06	0.3 (.1–.9)	.03	1.1 (.4–3.0)	.80		
Black race	0.8 (.4–2.0)	.67			0.8 (.3–2.2)	.71		
Combination ART	1.7 (.5–6.0)	.41			1.7 (.5–6.4)	.43		
HIV RNA <400 copies/mL	1.6 (.6–4.7)	.36			2.1 (.7–6.4)	.20		
Preentry 2009 seasonal influenza vaccination	0.3 (.1–.9)	.03			0.6 (.2–1.5)	.26		
Age (y)	1.0 (1.0–1.1)	.48			1.0 (.9–1.1)	.78		
CD4 count	1.0 (1.0–1.0)	.76			1.0 (1.0–1.0)	.11		
CD4%	1.0 (1.0–1.1)	.70			1.0 (1.0–1.1)	.15		
CD4 count ≥200 cells/μL	1.4 (.2–10.3)	.75			3.2 (.4–24.1)	.26		
CD4 count ≥500 cells/μL	1.5 (.6–4.1)	.43			2.8 (1.0–7.8)	.05		
CD4% ≥15	1.3 (.2–10.0)	.77			1.9 (.3–12.4)	.48		
CD8 count	1.0 (1.0–1.0)	.52			1.0 (1.0–1.0)	.25		
CD8%	1.0 (1.0–1.0)	.83			1.0 (1.0–1.0)	.87		
CD19 count	1.0 (1.0–1.0)	.47			1.0 (1.0–1.0)	.98		
CD19%	1.0 (.9–1.1)	.61			1.0 (.9–1.0)	.30		
Log_{10} baseline HAI titer	34.9 (4.0–307.9)	.001	68.7 (6.5–731.6)	.0005	2.3 (.9–5.9)	.07	16.3 (1.3–201.2)	.03

Abbreviations: AOR, adjusted odds ratio; ART, antiretroviral therapy; CI, confidence interval; HAI, hemagglutination inhibition; HIV, human immunodeficiency virus; OR, odds ratio.

[a] Limited to participants with baseline HAI titers <40.

[b] *P* value from univariate regression analysis.

[c] *P* value from multivariable regression analysis.

(*P* = .0002). Complete response was greater following first and second vaccinations among participants with baseline HAI titers ≥10 (Table 4).

Persistence

Seven months after the first vaccination, 57.2% of the participants demonstrated seroprotection. Seventy-three of the 113 (64.6%) participants with seroprotection after the second vaccination maintained HAI titers ≥40 seven months after first vaccination; the rates were 44.7% (17 of 38), 86.5% (32 of 37), and 63.2% (24 of 38) in the 4–8-, 9–17-, and 18–24-year age groups, respectively.

Correlates of Primary Response

Baseline factors associated with complete vaccine response (seroresponse and seroprotection) after first vaccination in univariate analysis among participants with baseline HAI titers <40 included baseline log_{10} HAI titers, Hispanic ethnicity, and receiving TIV prior to study vaccine (*P* < .1, Table 5). Only Hispanic ethnicity (adjusted odds ratio [AOR] = 0.3 [95% CI, .1–.9]; *P* = .03) and baseline log_{10} HAI (AOR = 68.7 for a 1 log_{10} increase [95% CI, 6.5–731.6]; *P* = .0005) were significant predictors of complete vaccine response in multivariable logistic regression analysis.

Correlates of Secondary Response

Following second vaccination, log_{10} baseline HAI titer (odds ratio [OR] = 8.0 for a 1 log_{10} increase [95% CI, .8–76.4]; *P* = .07; Table 5) and CD4 count ≥500 cells/μL (OR = 2.8 [95% CI, 1.0–7.8]; *P* = .05) were associated with complete vaccine response (both seroprotection and seroresponse). However, in multivariable analysis, only log_{10} baseline HAI titers remained predictive (AOR = 16.3 for a 1 log_{10} increase [95% CI, 1.3–201.2]; *P* = .03).

DISCUSSION

In this study we demonstrated the safety and immunogenicity of 2 vaccinations with high-dose pH1N1 antigen in perinatally HIV-1–infected children and young adults. Although a substantial portion of the participants had seroprotective levels of antibody (HAI titers ≥40) at baseline, the rate increased to 82.4% after 2 vaccinations. Additionally, in those without seroprotection at baseline, the seroprotection was 59.3% and 73.6% after the first and second vaccinations, respectively. Of those participants with HAI titers <40 after the first vaccination, 40.5% achieved seroprotection (HAI titers ≥40) after the second vaccination. We also demonstrated an improved seroresponse after the second vaccination: from 68.6% of

participants after the first vaccination to 82.4% after the second and in 46.5% of those who did not demonstrate seroresponse after first vaccination. In addition, the second vaccination resulted in significantly more complete responders (both seroresponse and seroprotection) than after 1 vaccination ($P = .0002$).

The levels of seroprotection after pH1N1 vaccination demonstrated in our population remain substantially lower than the seroprotection rates reported for HIV-uninfected children (85%–99%) in studies using a variety of inactivated vaccines, antigen doses, and vaccination schedules [27–35]. Similar to our findings in perinatally HIV-1–infected children and youth, 2 dose series yielded higher seroprotection rates in HIV-uninfected children [28, 31, 33, 34]. The current study regimen of 2 doses of 30 μg antigen per dose, when evaluated in healthy children, resulted in seroprotection rates ranging from 87.7% to 100%, depending on the population and vaccine [31, 33, 34].

The lower seroprotection rates found in our population compared with those in healthy children and youth receiving the pH1N1 vaccine are not surprising. Poor response to TIV in HIV-1–infected individuals has been previously demonstrated [9–16], though often associated with advanced disease states [12–15]. Of interest, the response of HIV-infected children (similar to our population) to a single dose of live, attenuated TIV was better than in the current study, demonstrating that 96%–100% of the participants achieved seroprotection for influenza A and 81%–88% for influenza B [36].

Seroprotection in HIV-infected adults following the recommended single dose of 15 μg antigen, unadjuvanted vaccine was achieved in only 54%–69% of participants [37–39]. Seroprotection rates reached 72.5%–88% after 1 dose [40–43] and 91%–97% following 2 doses of adjuvanted vaccine [38–40]. Experience with pH1N1 adjuvanted vaccines in HIV-infected children is limited to MF59 adjuvanted vaccine where seroprotection was achieved in 94%–100% and in all participants after 2 doses [43, 44].

Improved vaccine response in HIV-infected children with better immunologic status on cART has been demonstrated with hepatitis A and pneumococcal vaccines [45, 46]. However, we were unable to demonstrate an independent relationship between immune status at study entry and vaccine response, likely because our cohort was immunologically robust with a median CD4 count >500 cells/μL in the 2 older age groups and >1000 cells/μL in the youngest group. In addition, the median viral load was <100 copies/mL and only 6.4% participants were not receiving antiretroviral therapy. In contrast to our findings, studies of pH1N1 vaccination in HIV-infected adults demonstrated reduced response in those with lower baseline or nadir CD4 counts [39, 40, 43], longer duration of HIV infection [38, 40], or older age [38, 43]. There are differences in nadir CD4, duration of HIV, and age in perinatally infected children compared with adults because CD4 count is usually higher in young children and age correlates with duration of illness, thus possibly explaining the different findings.

Higher log-transformed baseline HAI titers were an independent predictor of improved primary and secondary complete response (both seroresponse and seroprotection). We do not know if higher $\log_{10}$ baseline HAI represented prior exposure to pH1N1 infection or cross-reaction with similar H1N1 antigens encountered in prior influenza vaccines or infections. We believe this demonstrated the benefit of antigenic boost and further supports the possible use of a 2-vaccine series for perinatally HIV-1–infected children and youth. Of interest, Hispanic ethnicity resulted in a lower rate of complete response following the first vaccination in the current study, which was not reported in other studies [27]. This relationship, however, was not seen following the second vaccination.

Consistent with data from prior studies and the Vaccine Adverse Event Reporting System [47], pH1N1 vaccination was safe in our population of HIV-1–infected children and youth, even at increased doses. Adverse events were few and mild in severity. No seizures were reported. Of interest are the 2 cases of herpes virus reactivation following vaccination, 1 with a concomitant facial nerve palsy, which has previously been reported as possibly related to seasonal influenza vaccine [48].

Our study had several limitations. Our baseline HAI titers were elevated; therefore, presumably, 32.6% of our participants were previously exposed to pH1N1 although there was no history of compatible symptoms. Our study did not initiate vaccination until the fall of 2009 and in most study sites; pH1N1 infection peaked in August 2009. This was a common finding in many other pH1N1 vaccine studies [27, 42, 43, 49]. Interestingly, Kok et al [50] assessed pre-pandemic serum samples from patients demonstrating seroprotection after the pandemic. Among the 34.2% of HIV-infected individuals demonstrating seroprotection following the pandemic, 12.8% had protective antibody levels prior to the pandemic, suggesting that cross-reacting antibodies may be present [50].

Additionally, we did not include groups of perinatally HIV-1–infected children and youth who received a single 15-μg dose or two 15-μg doses of pH1N1 vaccine. We did, however, conduct a companion study, P1089, that recruited perinatally HIV-1–infected children and youth, 6 months to 24 years of age, who were scheduled to receive 1 of the following commercially available pH1N1 vaccines: FluMist (MedImmune), Fluvirin (Novartis), or Fluzone (Sanofi Pasteur). In this nonrandomized evaluation, 93 participants in the present study who were 10 years to 24 years of age and received 2 high-dose vaccinations were compared to 50 P1089 participants who received the recommended 15-μg single dose.

Baseline demographics and seroprotection rates in P1088 (36.6%) and P1089 (42%) were similar ($P = .52$). No difference in seroprotection rates between studies was observed after the

first vaccination (P1088, 76.9% and P1089, 75%; $P = .80$). The seroprotection rate after the second vaccination in P1088 (84%) was comparable to that following a single vaccination in P1089 (75%) ($P = .22$). Seroresponse rates were also similar following first vaccination (P1088, 68.1% and P1089, 62.5%; $P = .50$). However, participants in P1088 demonstrated greater seroresponse rates after the second vaccination compared with P1089 participants after the single standard-dose vaccination (84% vs 62.5%; $P = .005$).

In conclusion, we have demonstrated the safety and immunogenicity of 2 doses of 30-μg pH1N1 antigen in HIV-1–infected children and youth. A substantial proportion of children who failed to respond to the first vaccine dose achieved seroprotection and seroresponse after the second dose. In response to new influenza pandemics, a 2-increased-dose vaccine series may provide improved protection against this infection.

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