CLINICAL AND VACCINE IMMUNOLOGY, Sept. 2011, p. 1503–1509 Vol. 18, No. 9
1556-6811/11/$12.00 doi:10.1128/CVI.05200-11

Long-Term Immunogenicity after One and Two Doses of a Monovalent MF59-Adjuvanted A/H1N1 Influenza Virus Vaccine Coadministered with the Seasonal 2009-2010 Nonadjuvanted Influenza Virus Vaccine in HIV-Infected Children, Adolescents, and Young Adults in a Randomized Controlled Trial▽

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Received 1 June 2011/Returned for modification 27 June 2011/Accepted 14 July 2011

Few data are available on the safety and long-term immunogenicity of A/H1N1 pandemic influenza vaccines for HIV-infected pediatric patients. We performed a randomized controlled trial to evaluate the safety and long-term immunogenicity of 1 versus 2 doses of the 2009 monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine (PV) coadministered with the seasonal 2009–2010 trivalent nonadjuvanted influenza vaccine (SV) to HIV-infected children, adolescents, and young adults. A total of 66 HIV-infected patients aged 9 to 26 years were randomized to receive one (group 1) or two (group 2) doses of PV coadministered with 1 dose of SV. The main outcome was the seroconversion rate for PV at 1 month. Secondary outcomes were the geometric mean titer ratios and the seroprotection rates at 1 month for all vaccines, seroconversion rates at 1 month for SV, and longitudinal changes of antibody titers (ABTs) at 1, 2, 6, and 12 months for all vaccines. Groups 1 and 2 had similar CD4 counts and HIV RNA levels during the study. The seroconversion rate for PV was 100% at 1 month in both groups. ABTs for PV were high during the first 6 months and declined below seroprotection levels thereafter. Longitudinal changes in ABTs were similar in groups 1 and 2 for both PV and SV. The side effects of vaccination were mild and mostly local. In HIV-infected children, adolescents, and young adults, the immune response triggered by a single dose of PV was similar to that obtained with a double dose and was associated with long-term antibody response.

In April 2009, a novel H1N1 influenza A virus was isolated in Mexico and in the United States, and its rapid worldwide diffusion led the World Health Organization to declare a new influenza pandemic within just 2 months (8). The rate of 2009 A/H1N1 infection was four times greater in children than in adults, and immunosuppressed individuals had a more severe course of the disease (8, 15). In September 2009, the Italian Ministry of Health recommended vaccination against 2009 A/H1N1 to all HIV-infected patients. In the meantime, the European Medicines Agency (EMA) issued a marketing authorization for two vaccines against 2009 A/H1N1 and allowed their administration together with the seasonal influenza vaccine.

Two phase-2 randomized controlled trials have shown that a single dose of 2009 pandemic A/H1N1 influenza vaccine is sufficiently immunogenic except for children younger than 9 years (18). Protection against influenza is provided mainly by antibody-mediated immunity, and HIV infection is associated with a decline in the number and function of antigen-specific memory B-cells that might hamper the response to vaccination (17). Owing to the novelty of the 2009 A/H1N1 infection and the uncertain response of HIV-infected children to vaccination, it was hypothesized that special vaccination schedules might be necessary in this population (21).

We performed a randomized controlled trial (RCT) to assess the safety and long-term immunogenicity of one versus two doses of the monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine coadministered with the seasonal 2009–2010 trivalent nonadjuvanted influenza vaccine to HIV-infected children, adolescents, and young adults.

MATERIALS AND METHODS

Study design. An RCT was performed between 15 October 2009 and 30 November 2010 to assess the long-term immunogenicity of the monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine coadministered with the seasonal 2009–2010 nonadjuvanted influenza vaccine.

Vertically HIV-infected children and adolescents followed as outpatients at the pediatric clinic of the L. Sacco Hospital (Milan, Italy) were studied. Eligible patients were aged 9 to 26 years and had received a seasonal influenza vaccine in the previous influenza season. Exclusion criteria were (i) body temperature ≥ 38°C at the time of vaccination, (ii) ongoing or recent immunosuppressive treatment, (iii) blood transfusions or use of intravenous immunoglobulins during the previous month, and (iv) influenza-like illness during the previous month.

Sixty-six consecutive HIV-infected patients were randomly assigned to receive one (group 1) or two (group 2) doses of the monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine coadministered with a dose of the seasonal 2009–2010 nonadjuvanted influenza vaccine. A second dose of the pandemic vaccine was administered only to group 2 within 28 ± 5 days from the first dose.

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▽ Published ahead of print on 27 July 2011.

A computer-generated randomization list assigned participants in equal numbers to group 1 ($n = 33$) or group 2 ($n = 33$). A statistician who did not perform the final analysis generated the allocation sequence and assigned participants to the treatment groups. The study was approved by the Ethical Committee of the L. Sacco Hospital (Milano, Italy), and written informed consent was obtained from the parents or legal guardians of the children and from the patients themselves.

Assessment of immunological and virological status. CD4 cell counts and HIV RNA levels were measured at baseline and at 2 (56 ± 5 days), 6 (168 ± 10 days), and 12 (336 ± 5 days) months after enrollment. CD4 cells were measured by flow cytometry using fresh blood samples and a Cytotron Absolute flow cytometer (Ortho Cytometry, Raritan, NJ) with Immunocount II software. HIV RNA was measured with a lower detection limit of 50 copies/ml (Quantiplex assay 3.0; Bayer Diagnostics).

Vaccines. The pandemic vaccine was a monovalent 2009 A/H1N1 pandemic influenza vaccine containing 7.5 μg of the hemagglutinin (HA) antigen of an A/California/7/2009(H1N1)-like strain (NYMC X-179A) adjuvanted with oil-in-water emulsion MF59 (Focetria; Novartis Vaccine, Italy).

The seasonal influenza vaccine was the 2009–2010 trivalent inactivated non-adjuvanted influenza vaccine containing 15 μg of HA antigen of the following strains: an A/Brisbane/59/2007(H1N1)-like strain (IVR-148), an A/Brisbane/10/2007(H3N2)-like strain (NYMC X-175C), and a B/Brisbane/60/2008-like strain (Influvac; Solvay Biologicals BV, The Netherlands).

Vaccines were supplied in prefilled monodose syringes containing 0.5 ml of the relevant vaccine formulation. The pandemic vaccine was injected in the left deltoid muscle and the seasonal vaccine in the right deltoid muscle. Each patient remained under observation for at least 30 min after vaccination.

Evaluation of antibody response to vaccination. Serum samples were collected at enrollment and 1 (28 ± 5 days), 2 (56 ± 5 days), 3 (74 ± 5 days), 6 (168 ± 10 days), and 12 (336 ± 5 days) months after vaccination. All samples were kept at −20°C until further analysis. Antibody titers (ABTs) against pandemic and seasonal influenza strains were measured using the HA inhibition (HI) test (24). The HI assay was performed using turkey erythrocytes and the relevant vaccine strains as antigens [A/California/7/2009(H1N1), A/Brisbane/59/2007(H1N1), A/Brisbane/10/2007(H3N2), and B/Brisbane/60/2008]. Specimens from each time point were measured in duplicate. The HI ABTs were expressed as the reciprocal of the highest dilution that inhibited agglutination. ABTs below the detection limit of 1:10 were assigned a value of 1:5.

Evaluation of vaccine safety. A detailed clinical history and physical examination were performed at enrollment. Diaries were provided to each patient or to her or his legal tutor to report the occurrence of local (erythema, swelling, induration, and pain) or systemic (axillary temperature ≥ 38.5°C, headache, malaise, fatigue, myalgia, arthralgia, shivering, or rash) side effects for 2 weeks after vaccination. Adverse events were defined as injuries or ailments related to or caused by the treatments under study. At each visit, patients or their legal tutors were specifically asked about adverse events, and the first author checked for any association between adverse events and morbidities.

Postvaccination influenza surveillance. Postvaccination influenza surveillance was performed from November 2009 to April 2010. Participants or their legal tutors were instructed to contact the study staff in the event of influenza-like illness. The latter was defined as an abrupt onset of fever (≥38.0°C) with one or more respiratory symptoms (nonproductive cough, sore throat, or rhinitis) and one or more systemic symptoms (myalgia, headache, or severe malaise). Patients with such symptoms were instructed to return immediately to the clinic to undergo further clinical assessment and for sampling using a nasal swab to exclude the possibility of pandemic or seasonal influenza. The virological diagnosis of influenza was performed using a one-step real-time reverse transcriptase (RT) multiplex PCR assay as described elsewhere (24).

Outcomes. The main outcome was the seroconversion rate to A/California/7/2009(H1N1) after 1 month of vaccination, i.e., the percentage of seronegative subjects with an ABT of A/California/7/2009(H1N1) ≥ 1:40 at 1 month and the percentage of seropositive subjects with at least a 4-fold increase in ABTs to A/California/7/2009(H1N1) at 1 month. With the assumption of a 1-month seroconversion rate to A/California/7/2009(H1N1) for 99% in patients receiving two doses of pandemic vaccine, examination of 30 subjects per group ensured a power of 93% to detect a between-group difference of 30% as significant at an alpha level of 0.05, corresponding to a seroconversion rate of 69% for the group receiving one dose of pandemic vaccine (Fisher's exact test). We estimated a 10% loss at follow-up, so we enrolled 33 subjects per group. Secondary outcomes were (i) the geometric mean titer ratios (GMTR) of antibodies to pandemic and seasonal vaccines at 1 month, (ii) the seroconversion rates for seasonal vaccines at 1 month, and (iii) the seroprotection rates for all vaccines. Seroprotection was defined as the percentage of vaccines with an ABT ≥ 1:40. Together with the main outcome, these secondary outcomes were used to evaluate immunogenicity (6). For a further outcome, we studied the changes in ABTs during 12 months using mixed linear regression models (see "Statistical analysis" below).

TABLE 1. Demographic data and clinical characteristics of the two study groups at baseline[a]

Characteristic	Group 1 ($n = 33$)	Group 2[b] ($n = 33$)
Sex (no. male/no. female)	15/18	15/18
Age (years)	20 (9)	19 (9)
CD4 T cells (cell/mm^3)	782 (403)	652 (478)
CD4 T cells (%)	35 (11)	33 (14)
No. of subjects with HIV RNA < 50 copies/μl	27/33	28/33

[a] Group 1 consisted of patients administered 1 dose of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a seasonal vaccine. Group 2 consisted of patients administered 2 doses of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a seasonal vaccine. Values in parentheses represent median and interquartile ranges for continuous variables and numbers of subjects for categorical variables.

[b] One patient was lost during the follow-up and did not receive the second dose of the pandemic vaccine, and another refused to undergo the second dose.

Statistical analysis. Age and CD4 cells were not normally distributed and are reported as median and interquartile ranges (IQR). Categorical variables are reported as the numbers or percentages of subjects with the characteristic of interest. Between-group comparisons of categorical variables were performed using Fisher's exact test. The main and secondary outcomes are described below (see "Study design").

The longitudinal changes of ABTs were evaluated using mixed linear regression models employing (i) $\log_2$ ABTs as the outcome; (ii) the treatment group (category 0, one-dose group; category 2, two-dose group), month (categories 0, 1, 2, 3, 6, and 12 months), a treatment × month interaction (category × category), and basal $\log_2$ ABT as covariates; and (iii) the patient outcomes codified as random effects (12). To check model fit, we tested whether random intercepts were normally distributed using kernel density plots and the Shapiro-Wilk test. Although the plots and the Shapiro-Wilk test results suggested violation of normality only for the random intercept of the B/Brisbane/60/2008 model, we calculated confidence intervals (CIs) using the percentile bootstrap method (10,00 samples) for all models in order to relax the assumption of homoscedasticity of random effects (8, 19). Using this approach, we compared the time points of the curves representing the ABT-time relationship for the two study groups. Statistical analysis was performed using Stata 11.1 software (Stata Corp., College Station, TX).

RESULTS

Table 1 shows the demographic and clinical characteristics of the study population. Sixty-six HIV-infected patients aged 9 to 26 years, with a median age of 19 years (IQR, 9 years), were randomly assigned to group 1 ($n = 33$) or group 2 ($n = 33$). Group 1 and group 2 had similar characteristics at enrollment. Sixty-two patients were undergoing highly active antiretroviral therapy (HAART), 3 patients had never received any kind of HAART, and 1 patient had stopped HAART. Twenty-eight patients were in CDC class A (mildly symptomatic), 17 in class B (moderately symptomatic), 18 in class C (severely symptomatic), and 3 in class N (asymptomatic). At enrollment, 55 patients had HIV RNA levels < 50 copies/ml; 15 patients had CD4 counts of 200 to 500 cells/μl and 51 of more than 500 cells/μl.

One patient randomized to group 2 was lost to follow-up and did not receive the second dose of the pandemic vaccine. Another group 2 patient refused to undergo the second dose. Thus, 34 patients in group 2 were actually available for analysis. During the study, the members of groups 1 and 2 showed similar CD4 counts and viral loads (Fig. 1). Among the 11 patients with detectable HIV RNA levels at enrollment, 6

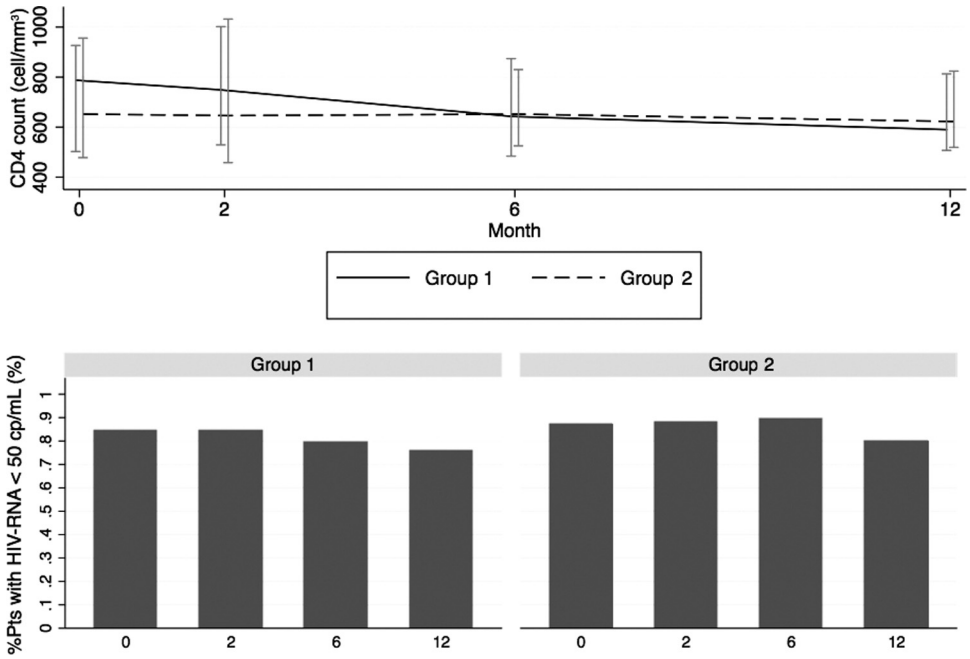

FIG. 1. CD4 counts and percentages of patients (Pts) with HIV RNA < 50 copies/ml during the study in group 1 (1 dose of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a dose of seasonal vaccine) and group 2 (2 doses of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a dose of seasonal vaccine). Values represent medians and interquartile ranges (IQR) for continuous variables and percentages of subjects for categorical variables.

belonged to group 1 and 5 to group 2. Two of the group 2 patients with detectable HIV RNA levels were those lost to follow-up. Two patients, with baseline values of 41,500 cp/ml and 43,340 cp/ml, showed a decrease of at least 1 log unit at 12 months. A viremia level of 67,000 cp/ml remained virtually stable in a patient. Two patients with baseline values of 127 cp/ml and 110 cp/ml had undetectable levels of HIV RNA at 12 months. The remaining four patients, with baseline values of 60,900 cp/ml, 18,300 cp/ml, 1,786 cp/ml, and 153 cp/ml, showed an increase of less than 1 log unit at 12 months.

About 3% of group 1 and group 2 patients had an ABT ≥ 1:40 against A/California/7/2009(H1N1) at enrollment. One month after vaccination, this percentage rose to 100% in both groups. The seroconversion rate against A/California/7/2009 (H1N1) at 1 month (main outcome) was the same in group 1 and group 2 ($P = 0.190$; Fisher's exact test). One month after the first vaccination, the GMTR for A/California/7/2009 (H1N1) was high in both groups (Table 2). The fact that the GMTR for A/California/7/2009(H1N1) was higher for group 1 rather than for group 2 is likely to have been a matter of chance, because this was a randomized trial and controlling for the basal value of A/California/7/2009(H1N1) in mixed linear regression analysis had no effect on the time changes of the relative ABT values (see below). Such immune responses met the EMA criteria for vaccine immunogenicity, with rates of seroconversion and seroprotection of 100% in both groups. Although the study was underpowered for detection of differences < 30%, between-group differences were low and of no clinical relevance. The percentage of subjects who developed a protective ABT against seasonal antigens was >90% at 1 month for both groups. The GMTRs for such antigens ranged from 12 (B antigen) to 23 (A/H1N1 antigen). Thus, the immune response to seasonal antigens met EMA criteria for licensing in both groups (a ≥4-fold increase in HI antibody titers and a titer of ≥1:40 in >40% of subjects, an HI antibody titer of ≥1:40 reached in >70% of subjects, and a >2.5-fold increase in geometric mean titer of HI antibodies).

Last, we evaluated the mean (standard deviation [SD]) changes in the $\log_2$ ABTs for A/California/7/2009(H1N1), A/Brisbane/59/2007(H1N1), A/Brisbane/10/2007(H3N2), and B/Brisbane/60/2008 (Fig. 2). ABT changes with time were virtually identical in the two study groups. The only statistically significant difference, detected at month 2 for A/California/7/2009(H1N1) (0.7 $\log_2$ units [95% CI, 0.2 to 1.2]), can be dismissed as clinically irrelevant.

In order to test whether the viral load or CD4 count was associated with the response to vaccination, we performed a secondary analysis, adding a covariate coding for HIV RNA suppression (0, no; 1, yes) or for CD4 counts (in cells per microliter) to the regression model described above.

Because both HIV RNA and CD4 cell results were evaluated at 0, 2, 6, and 12 months, we could not test the effect of the changes in these parameters on ABTs at 1 month. These analyses showed that neither HIV RNA suppression nor CD4 counts were associated with any ABTs at the available time points (data not shown) and that baseline HIV RNA suppression and CD4 counts were not associated with ABTs at 1 month. The secondary analyses should be considered only explorative, because (i) they were not preplanned, (ii) there were very few subjects with detectable HIV RNA levels, and (iii) the effect of HIV RNA and CD4 cells on the response to vaccination can be properly evaluated only by including them in a stratified randomization procedure.

The percentages of seroprotected patients detected during

TABLE 2. Seroconversion rate 1 month after treatment with the seasonal influenza vaccine coadministered with the first dose of pandemic vaccine in HIV-infected children, adolescents, and young adults[a]

Strain	Group 1 (n = 33) (95% CI)				Group 2 (n = 33) (95% CI)			
	GMT0	GMTR1	SCV1	SPT1	GMT0	GMTR1	SCV1	SPT1
A/Cal/7/09	5.8 (4.8–7.0)	85.8 (62.4–100.0)	100.0 (91.3–100.0)	100.0 (91.3–100.0)	5.8 (4.8–7.2)	48.9 (32.3–74.0)	100.0 (90.8–100.0)	100.0 (90.8–100.0)
A/Br/59/07	11.1 (7.3–6.9)	22.9 (14.5–35.9)	97.0 (90.7–100.0)	100.0 (91.3–100.0)	12.2 (7.5–19.9)	20.0 (11.5–34.5)	87.0 (74.5–99.6)	96.8 (85.1–99.8)
A/Br/10/07	8.6 (6.2–12.0)	18.6 (11.9–28.8)	93.9 (85.3–100.0)	93.9 (81.4–99.0)	9.1 (6.6–12.6)	20.0 (12.8–32.2)	93.5 (84.3–100.0)	96.8 (85.1–99.8)
B/Br/60/08	7.5 (5.8–9.5)	11.6 (7.8–17.4)	90.9 (80.5–100.0)	93.9 (81.4–99.0)	7.0 (5.4–9.1)	12.8 (9.4–17.3)	96.7 (90.2–100.0)	96.8 (85.1–99.8)

[a] Group 1 consisted of patients administered 1 dose of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a seasonal vaccine. Group 2 consisted of patients administered 2 doses of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine plus a seasonal vaccine. Abbreviations: GMT0, geometric mean titer at baseline; GMTR1, ratio between the GMT at 1 month (GMT1) and baseline GMT0; SCV1, seroconversion at 1 month; SPT1, seroprotection at 1 month.

the study are depicted in Fig. 3. The percentage of protective ABTs against A/Cal/7/09 rose to nearly 100% after 1 month in both groups, and only minor changes were observed thereafter. The percentages of protective ABTs against A/California/7/2009(H1N1) were high during the first 6 months but started to decline thereafter, reaching 49% and 64% at 12 months in group 1 and group 2, respectively. The percentages of protective ABTs against A/Brisbane/59/2007(H1N1) and A/Brisbane/10/2007(H3N2) were high (>75%) during the first 3 months in both groups and declined only for B/Brisbane/60/2008. Twelve months after vaccination, the percentages of protective ABTs were about 70% for A/Brisbane/59/2007(H1N1), 50% for A/Brisbane/10/2007(H3N2), and less than 40% for B/Brisbane/60/2008. At 12 months, the seroprotection rate was <70% for all seasonal antigens.

Safety assessment. Side effects were mostly local, and all were mild (Table 3). In group 1, 15 patients reported 29 possible adverse events and all were mild. In group 2, 8 possible adverse events were reported by 5 patients and all were again mild. All adverse events resolved spontaneously within 7 days. The most common adverse event was mild pain at the site of injection. Headache and malaise for 1 to 2 days were the most common systemic reactions. No patients reported influenza-like illness during the postvaccination surveillance.

DISCUSSION

We found that HIV-infected children, adolescents, and young adults are able to generate and maintain a long-lasting antibody response to the 2009 monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine. Our results also suggest that one dose of such vaccine is comparable to two doses in providing a good immunogenic response when administered to HIV-infected patients with good immune reconstitution. Similar findings were observed for seasonal influenza vaccine, showing that the trivalent seasonal vaccine and the pandemic 2009 A/H1N1 influenza vaccine can be coadministered safely and with a good immunogenic response.

This study has some limitations. Because most of our patients had good viral and immunological profiles and all were 9 years of age and older, we could not determine the impact of severe immunosuppression and of younger age on the response to 2009 A/H1N1 vaccination. Although a secondary analysis showed that neither HIV RNA nor CD4 counts were associated with the response to vaccination, only a specifically designed RCT could address this issue. Moreover, the microneutralization assay is expected to provide a better measurement of ABTs than the hemagglutination inhibition assay employed in this study.

Conflicting data are available on the immunogenicity of 2009 pandemic vaccines in HIV-infected adults. The immunogenicity of a single unadjuvanted inactivated split virus A/H1N1 vaccine was evaluated by Tebas et al., who concluded that up to 40% of HIV-infected individuals are not seroprotected after vaccination (22). Bickel et al. reported seroconversion rates of 68% and 92% after one and two doses of an AS03-adjuvanted 2009 pandemic influenza vaccine, respectively (3). Patients who failed to seroconvert had lower CD4 counts and a longer duration of HIV infection. Orlando et al. reported a serocon-

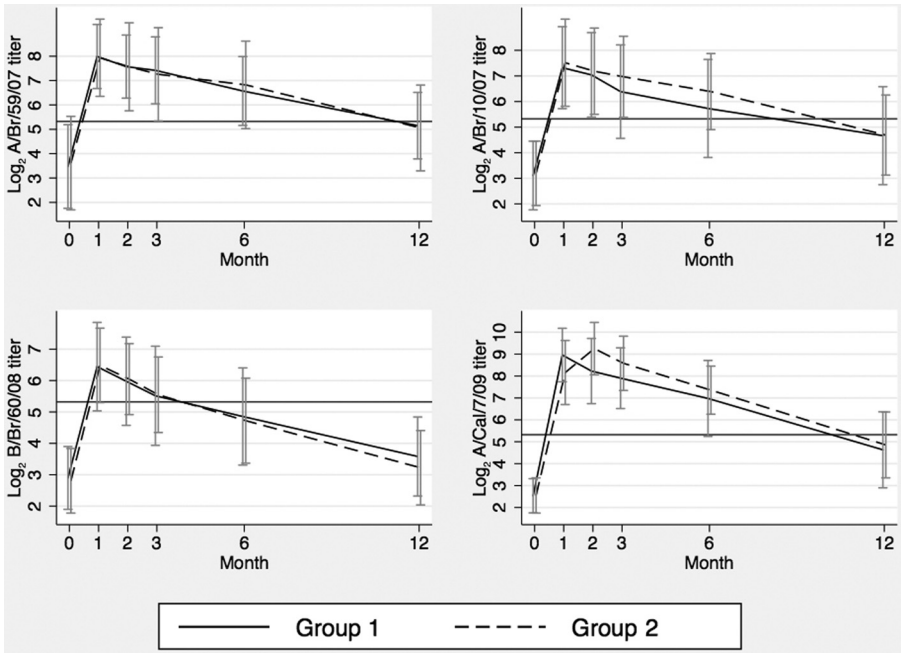

FIG. 2. Changes of antibody titers in group 1 (1 dose of monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine plus a dose of 2009–2010 seasonal unadjuvanted vaccine) and group 2 (2 doses of monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine plus a dose of 2009–2010 seasonal unadjuvanted vaccine). An offset of 0.1 was used to make standard deviations visible. A horizontal line in each panel marks the $\log_2$ value corresponding to a protective titer of 1:40 (5.3 $\log_2$ units).

version rate of 92% after a single shot of a monovalent 2009 A/H1N1 MF59-adjuvanted influenza vaccine (17). Seroprotection was 6 times more likely in patients with CD4 cells counts $\geq$ 500 cell/mm^3. Last, Soonawala et al. reported a seroprotection rate of 88% after one dose of MF59-adjuvanted A/H1N1 vaccine administered to HAART-treated HIV-infected adults with a median CD4 cell count of about 500 cells/mm^3 (20). The immunogenic response to 2009 pandemic influenza vaccine of HIV-infected patients was similar to that of healthy controls, and a second vaccination did not increase immunogenicity.

Few data on the immunogenicity of 2009 A/H1N1 vaccines for children are available. The immunogenicity of two doses of AS03-adjuvanted 2009 pandemic influenza vaccine (3.75 μg of HA versus 1.9 μg of HA) was evaluated for children aged 3 to 17 years in a recent study (13). In this study, a single dose of 3.75 μg of HA plus $AS03_A$ adjuvant was as effective as a single dose of 1.9 μg of HA plus $AS03_B$ adjuvant to satisfy the targets specified by the EMA Committee for Medical Products for Human Use. In a study of HIV-infected children, Esposito et al. evaluated the immunogenicity and safety of the 2009 A/H1N1 MF59-adjuvanted influenza vaccine administered sequentially or simultaneously with the seasonal 2009-2010 virosome-adjuvanted influenza vaccine (11). In this study, seroprotection and seroconversion rates against the 2009 pandemic influenza virus were both 100% 2 months after administration to both HIV-infected and control children, regardless of the sequence of administration. Interestingly, the immune response to the pandemic and seasonal vaccines was greater when the vaccines were administered simultaneously and coadministration with virosome-adjuvanted seasonal antigens seemed to increase the GMTs for both pandemic and seasonal viruses. In our study, seroprotection and seroconversion rates of 100% against 2009 pandemic influenza were observed at 1, 2, and 3 months after vaccination, regardless of the dose employed. Our results confirm the high immunogenic response to one dose of the 2009 pandemic influenza adjuvanted vaccine reported in studies of healthy children and adolescents as well as HIV-infected children and adults (11, 13, 17).

It should be noted, however, that the good immune response of our HIV-infected children is at odds with that reported by some studies carried out with adults (3, 22). A possible explanation for this difference is that the degree of immune reconstitution and the type of vaccine are determinants of the response to vaccination. A 3-year follow-up study has shown not only that HIV-infected individuals have lower ABTs than healthy controls but also that their response to seasonal vaccination is inversely associated with CD4 count (5). The immune reconstitution of our patients was quite good for the duration of the study, and this might explain why one dose and two doses of pandemic vaccine were equally effective. Another possible explanation for our results is that the monovalent MF59-adjuvanted pandemic influenza vaccine has a better immunogenic profile than conventional nonadjuvanted vaccines, being especially advantageous for children, adults with chronic disease, elders, and immunocompromised patients (5, 9, 10, 14). Of course, another explanation for our findings is that the baseline seroprotection rate was very low (about 3%) in both study groups. A prevaccination ABT $>$ 1:40 is, in fact, inversely associated with the probability of seroconversion and seroprotection. As reported by Soonawala et al., a 4-fold increase in ABT was more difficult to achieve when the baseline titer was already high, while greater rates of

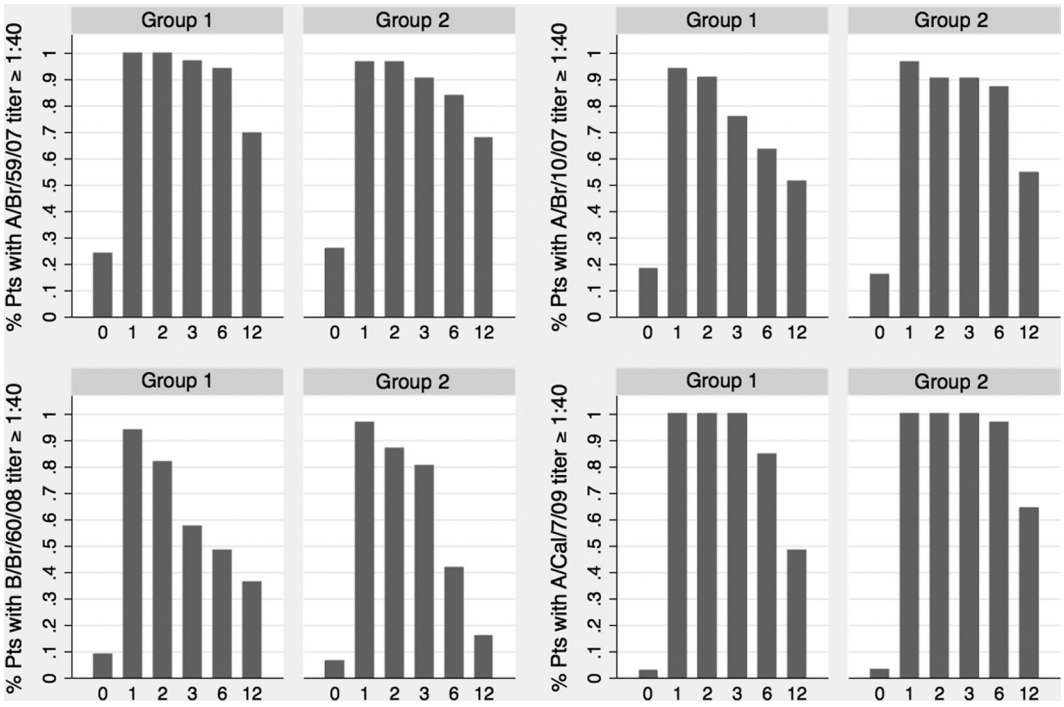

FIG. 3. Changes in seroprotection rates during the study in group 1 (1 dose of monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine plus a dose of 2009–2010 seasonal unadjuvanted vaccine) and group 2 (2 doses of monovalent 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine plus a dose of 2009–2010 seasonal unadjuvanted vaccine).

seroconversion were found for participants with undetectable ABTs at baseline (20).

The persistence of antibody response is important, because the influenza season often spans 6 months (20). Moreover, pandemic influenza may occur during nonseasonal months also, requiring prolonged immunity (21). Our report is the first to presenting data concerning the long-term duration of the antibody response to the monovalent MF59-adjuvanted 2009 pandemic vaccine among HIV-infected children and adolescents. Our data show that HIV-infected children and adolescents maintain protective ABTs against A/California/7/2009(H1N1) during the first 6 months after vaccination, regardless of the dose employed. In contrast, a recent study showed that HIV-infected adults are less likely than healthy adults to maintain a protective response to pandemic influenza vaccine for 6 months (7). In this study, younger age and receipt of HAART were associated with higher GMTs at 6 months.

The response of our HIV-infected patients to the 2009-2010

TABLE 3. Adverse events after the first dose of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine simultaneously administered with nonadjuvanted seasonal vaccine and after the second dose of monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine to HIV-infected children, adolescents, and young adults

Patient condition	Result after administration of indicated monovalent pandemic influenza A/H1N1 MF59-adjuvanted vaccine									
	First dose + seasonal vaccine (n = 66)					Second dose (n = 31)				
	n	%	Mild	Moderate	Severe	n	%	Mild	Moderate	Severe
Local										
Any	21	31.8	21	0	0	6	19.3	6	0	0
Pain	12	18.2	12	0	0	3	9.6	3	0	0
Erythema-swelling	5	7.6	5	0	0	2	6.4	2	0	0
Induration	4	6.1	4	0	0	1	3.2	1	0	0
Ecchymosis	0	0	0	0	0	0	0	0	0	0
Systemic										
Any	8	12.1	8	0	0	2	6.4	2	0	0
Fever	0	0.0	0	0	0	0	0.0	0	0	0
Headache	3	4.5	3	0	0	2	6.4	2	0	0
Malaise	3	4.5	3	0	0	0	0.0	0	0	0
Myalgia/arthralgia	2	3.0	2	0	0	0	0.0	0	0	0
Shivering	0	0.0	0	0	0	0	0.0	0	0	0
Rash	0	0.0	0	0	0	0	0.0	0	0	0

trivalent inactivated nonadjuvanted influenza vaccine was very good at 1 month, but a progressive decline of the seroprotection against B antigen occurred within 6 months. It is known that administration of B antigens with nonadjuvanted vaccines is associated with a lower immune response than the administration of A antigens (10, 23). Previous studies have shown that a trivalent inactivated influenza vaccine is associated with a similar time course of HI antibodies in HIV-infected children and age-matched healthy controls (1, 16).

Esposito et al. found that the simultaneous administration of two adjuvanted vaccines increased the GMTs to both pandemic and seasonal viruses (11). Our data show that a high ABT is obtained also after the simultaneous administration of inactivated nonadjuvanted influenza vaccines and MF59-adjuvanted pandemic vaccine. Moreover, the administration of one versus two doses of the pandemic vaccine did not affect the immune response to the seasonal vaccine either in terms of seroprotection/seroconversion rates or of ABT. The safety of the 2009 MF59-adjuvanted pandemic vaccine and that of the 2009-2010 seasonal influenza vaccines for children and adolescents has been reviewed recently, and our favorable safety data are in agreement with those findings (2, 4).

In agreement with other researchers (2, 16), we did not detect changes of viral load and CD4 cell counts after immunization. Moreover, we did not observe any difference in ABTs for both pandemic and seasonal vaccines between subjects with controlled viremia and those with uncontrolled viremia. However, as we pointed out in discussing the limitations of this study, the small number of patients with uncontrolled viremia made this an exploratory finding only.

In conclusion, the coadministration of the 2009 A/H1N1 pandemic influenza MF59-adjuvanted vaccine with an inactivated nonadjuvanted seasonal influenza vaccine to HIV-infected children, adolescents, and young adults was well tolerated and was associated with a long-term antibody response that was independent of the vaccine dose.

ACKNOWLEDGMENT

We have no conflicts of interest that are directly relevant to the content of this study.

REFERENCES

1. **Amendola, A., et al.** 2010. Influenza surveillance in a cohort of HIV-infected children and adolescents immunized against seasonal influenza. Vaccine **28:**2700–2704.
2. **Arguedas, A., et al.** 2011. Assessment of the safety, tolerability and kinetics of the immune response to A/H1N1v vaccine formulations with and without adjuvant in healthy pediatric subjects from 3 through 17 years of age. Hum. Vaccin. **7:**58–66.
3. **Bickel, M., et al.** 2011. Immune response after two doses of the novel split virion, adjuvanted pandemic H1N1 influenza A vaccine in HIV-1-infected patients. Clin. Infect. Dis. **52:**122–127.
4. **Black, S., et al.** 2010. Safety of MF59-adjuvanted versus non-adjuvanted influenza vaccines in children and adolescents: an integrated analysis. Vaccine **28:**7331–7336.
5. **Cagigi, A., A. Nilsson, S. Pensieroso, and F. Chiodi.** 2010. Dysfunctional B-cell responses during HIV-1 infection: implication for influenza vaccination and highly active antiretroviral therapy. Lancet Infect. Dis. **10:**499–503.
6. **de Carvalho, A. P., L. C. Dutra, and E. Tonelli.** 2003. Influenza vaccination in HIV infected children: immunologic and viral load changes. J. Pediatr. (Rio J.) **79:**29–40. (In Portuguese.)
7. **Crum-Cianflone, N. F., et al.** 2011. Durability of antibody responses after receipt of the monovalent 2009 pandemic influenza A (H1N1) vaccine among HIV-infected and HIV-uninfected adults. Vaccine **29:**3183–3191.
8. **Dawood, F. S., et al.** 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N. Engl. J. Med. **360:**2605–2615.
9. **Durando, P., et al.** 2008. Safety and immunogenicity of two influenza virus subunit vaccines, with or without MF59 adjuvant, administered to human immunodeficiency virus type 1-seropositive and -seronegative adults. Clin. Vaccine Immunol. **15:**253–259.
10. **Durando, P., G. Icardi, and F. Ansaldi.** 2010. MF59-adjuvanted vaccine: a safe and useful tool to enhance and broaden protection against seasonal influenza viruses in subjects at risk. Expert Opin. Biol. Ther. **10:**639–651.
11. **Esposito, S., et al.** 2011. Pandemic influenza A/H1N1 vaccine administered sequentially or simultaneously with seasonal influenza vaccine to HIV-infected children and adolescents. Vaccine **29:**1677–1682.
12. **Fitzmaurice, G. M., M. Davidian, G. Verbeke, and G. Molenberghs (ed.)** 2008. Longitudinal data analysis. CRC Press, Boca Raton, FL.
13. **Garcia-Sicilia, J., et al.** 2011. Immunogenicity and safety of AS03-adjuvanted H1N1 pandemic vaccines in children and adolescents. Vaccine **29:**4353–4361.
14. **Iorio, A. M., et al.** 2003. Antibody responses and HIV-1 viral load in HIV-1-seropositive subjects immunised with either the MF59-adjuvanted influenza vaccine or a conventional non-adjuvanted subunit vaccine during highly active antiretroviral therapy. Vaccine **21:**3629–3637.
15. **Kunisaki, K. M., and E. N. Janoff.** 2009. Influenza in immunosuppressed populations: a review of infection frequency, morbidity, mortality, and vaccine responses. Lancet Infect. Dis. **9:**493–504.
16. **Machado, A. A., et al.** 1 March 2011, posting date. Immunogenicity of an inactivated influenza vaccine and postvaccination influenza surveillance in HIV-infected and noninfected children and adolescents. AIDS Res. Hum. Retrovir. [Epub ahead of print.]
17. **Orlando, G., et al.** 2010. Pandemic influenza vaccine in adult HIV-1-infected patients. AIDS **24:**2142–2143.
18. **Plennevaux, E., E. Sheldon, M. Blatter, M. K. Reeves-Hoché, and M. Denis.** 2010. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. Lancet **375:**41–48.
19. **Rabe-Hesketh, S., and A. Skrondal.** 2008. Multilevel and longitudinal modeling using StataMultilevel and longitudinal modeling using Stata. Stata Press, College Station, TX.
20. **Soonawala, D., G. F. Rimmelzwaan, L. B. Gelinck, L. G. Visser, and F. P. Kroon.** 2011. Response to 2009 pandemic influenza A (H1N1) vaccine in HIV-infected patients and the influence of prior seasonal influenza vaccination. PLoS One **6:**e16496.
21. **Sutcliffe, C. G., and W. J. Moss.** 2010. Do children infected with HIV receiving HAART need to be revaccinated? Lancet Infect. Dis. **10:**630–642.
22. **Tebas, P., et al.** 2010. Poor immunogenicity of the H1N1 2009 vaccine in well controlled HIV-infected individuals. AIDS **24:**2187–2192.
23. **Walter, E. B., et al.** 2006. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? Pediatrics **118:**e570–e578.
24. **World Health Organization (WHO).** 2002. WHO manual on animal influenza diagnosis and surveillance. WHO document WHO/CDS/CSR/NCS/2002.5. World Health Organization, Geneva, Switzerland. http://www.who.int/vaccine_research/diseases/influenza/WHO_manual_on_animal-diagnosis_and_surveillance_2002_5.pdf.