MAJOR ARTICLE

A Single Dose of Unadjuvanted Novel 2009 H1N1 Vaccine Is Immunogenic and Well Tolerated in Young and Elderly Adults

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Background. When the novel H1N1 influenza A strain appeared in April of 2009, development of novel H1N1 vaccines became a public health priority.

Methods. We conducted a phase-2, multicenter, randomized, placebo-controlled, observer-blind clinical trial of a 2009 H1N1 vaccine in 1313 young (age, 18–64 years) and older (age, ⩾65 years) adults. Participants were randomized 1:4:4:4 to receive 2 doses of placebo or 7.5, 15, or 30 μg of H1N1 hemagglutinin administered 21 days apart. In post hoc analyses, hemagglutination inhibition (HI) titers measured at baseline and after vaccination were analyzed for young adults (age, 18–64 years), "younger elderly" adults (age, 65–74 years), and "very elderly" adults (age, ⩾75 years).

Results. At baseline, 28.8% of young adults, 43.9% of younger elderly adults, and 62.9% of very elderly adults had HI titers to A/2009 H1N1 of ⩾1:40. A single 7.5-μg dose induced HI titers ⩾1:40 in 94.5% (95% confidence interval [CI], 91.8%–96.3%) of all adults. After one 7.5-μg dose, the geometric mean titers achieved were 326.4 (95% CI, 275.9–386.0) in young adults, 155.4 (95% CI, 123.4–195.8) in "younger elderly" adults, and 243.9 (95% CI, 167.1–356.0) in "very elderly" adults.

Conclusions. This large phase-2 trial demonstrated that a single 7.5-μg dose of a monovalent unadjuvanted H1N1 vaccine induced protective HI antibody levels in adults of all ages, including very elderly adults.

Trial registration. Clinicaltrials.gov identifier NCT00958126

The rapid spread of a novel influenza A 2009 virus (2009 H1N1) led to the declaration of a pandemic in June 2009 [1]. As of May 2010, this virus had spread to >200 countries and caused >18,000 deaths [2], underscoring the need to develop and deploy safe and effective H1N1 vaccines.

Clinical trials of 2009 H1N1 vaccines were initiated during July and August 2009 [3–5]. Preliminary data from these studies suggested that one dose of unadjuvanted vaccine containing 15 μg of hemagglutinin antigen (HA) was sufficient for immunization of adults. On the basis of these studies, several pandemic 2009 H1N1 vaccines were licensed in the United States and other countries [6, 7]. Since that time, additional trials have shown that single unadjuvanted 7.5- and 11-μg HA doses of split-virus H1N1 vaccines are immunogenic in adults [8, 9].

Public health officials justifiably targeted pregnant women, children, and young adults in H1N1 vacci-

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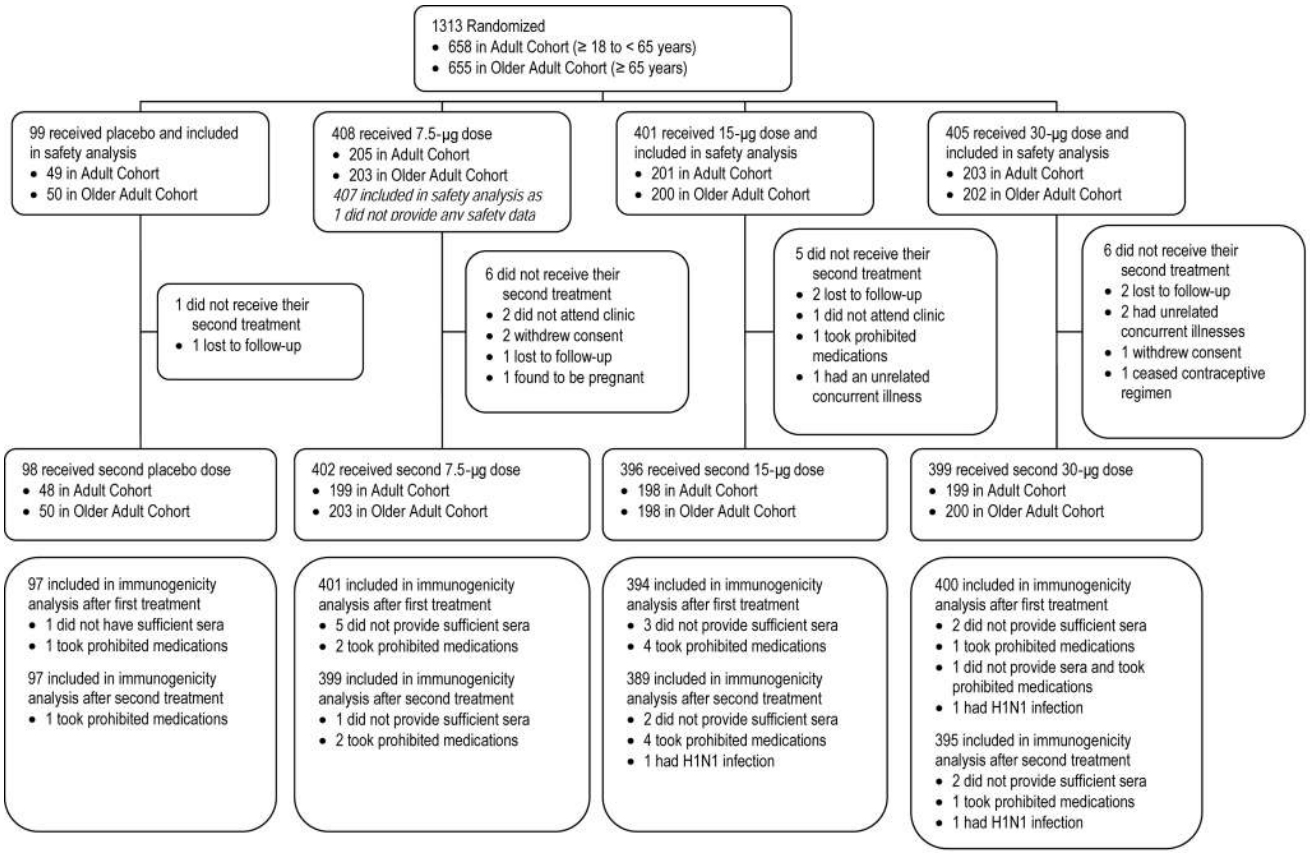

Figure 1. Enrollment and outcomes.

nation campaigns, because they were the groups most affected by this novel virus [10, 11]. However, outbreaks of 2009 H1N1 occurred in long-term care facilities [12], and the highest H1N1-associated case-fatality rate was reported in elderly individuals [13]. Because H1N1 is predicted to be the predominant circulating influenza strain in 2010 and is included in the 2010–2011 trivalent influenza vaccines for the Northern and Southern Hemispheres, it becomes increasingly important to assess antibody responses to 2009 H1N1 in individuals ⩾65 years of age.

The response of elderly individuals to 2009 H1N1 cannot necessarily be predicted based on previous studies of influenza vaccine. Many clinical trials have demonstrated diminished antibody responses to seasonal influenza vaccine in elderly adults, compared with young adults [14–17]; responses to 2009 H1N1 may follow this pattern. Alternatively, infection with an antigenically related virus in early life [18–21] may have primed elderly adults for an augmented antibody response. This might be particularly true for very elderly individuals ⩾75 years of age, many of whom were likely infected with H1N1 influenza viruses that circulated between 1918 and the 1930s and that are antigenically closely related to the H1N1 2009 virus [19].

We conducted a phase-2, dose-ranging, multicenter clinical trial of a monovalent, unadjuvanted split-virus 2009 H1N1 influenza vaccine (H1N1 vaccine) in adults. The prespecified objectives of this study were to assess the immunogenicity, safety, and tolerability of 7.5, 15, or 30 μg of the H1N1 vaccine in young and elderly adults. However, the large sample size also afforded the opportunity to compare hemagglutination inhibition (HI) antibody responses in younger (age, 18–64 years), younger elderly (age, 65–74 years), and very elderly (age, ⩾75 years) adults and to examine the effects of sex, race (black or white), and receipt of seasonal influenza vaccine in the preceding 12 months on antibody responses to the H1N1 vaccine.

METHODS

Study design. This phase-2, prospective, randomized, placebo-controlled, observer-blind parallel-group clinical study was conducted at 11 sites (Baltimore, Maryland; Rockville, Maryland; Metairie, Louisiana; Salt Lake City, Utah; San Diego, California; South Bend, Indiana; Peoria, Illinois; Austin, Texas; Melbourne, Florida; Huntsville, Alabama; and Fort Worth, Texas). The purpose of the present study was to evaluate, with the use of a 2-vaccination regimen, the immunogenicity and safety of 3 different doses of H1N1 vaccine in healthy adults

Table 1. Demographic Characteristics of the Subjects

Characteristic	Younger adults,[a] by dose received					Older adults,[b] by dose received					All subjects (*n* = 1312)
	Placebo (*n* = 49)	7.5 μg (*n* = 204)	15 μg (*n* = 201)	30 μg (*n* = 203)	All (*n* = 657)	Placebo (*n* = 50)	7.5 μg (*n* = 203)	15 μg (*n* = 200)	30 μg (*n* = 202)	All (*n* = 655)	
Age, years											
Mean ± SD	42.5 ± 12.7	42.6 ± 12.9	40.9 ± 13.1	40.8 ± 13.4	41.5 ± 13.1	70.9 ± 4.7	71.7 ± 5.5	71.2 ± 5.3	71.7 ± 5.6	71.5 ± 5.4	56.5 ± 18.0
Median (range)	40 (18–64)	44 (18–64)	41 (18–64)	41 (18–64)	41 (18–64)	69 (65–83)	71 (65–90)	70 (65–92)	70.5 (65–93)	70 (65–93)	64 (18–93)
Sex											
Male	18 (36.7)	81 (39.7)	88 (43.8)	90 (44.3)	277 (42.2)	21 (42.0)	83 (40.9)	92 (46.0)	90 (44.6)	286 (43.7)	563 (42.9)
Female	31 (63.3)	123 (60.3)	113 (56.2)	113 (55.7)	380 (57.8)	29 (58.0)	120 (59.1)	108 (54.0)	112 (55.4)	369 (56.3)	749 (57.1)
Race											
White	35 (71.4)	152 (74.5)	158 (78.6)	159 (78.3)	504 (76.7)	45 (90.0)	190 (93.6)	189 (94.5)	186 (92.1)	610 (93.1)	1114 (84.9)
Black	11 (22.4)	43 (21.1)	36 (17.9)	34 (16.7)	124 (18.9)	4 (8.0)	7 (3.4)	7 (3.5)	6 (3.0)	24 (3.7)	148 (11.3)
Other	3 (6.1)	9 (4.4)	7 (3.5)	10 (4.9)	29 (4.4)	1 (2)	6 (3)	4 (2)	10 (5)	21 (3.2)	50 (3.8)
Seasonal influenza vaccine[c] received	19 (38.8)	75 (36.8)	75 (37.3)	75 (36.9)	244 (37.1)	32 (64.0)	151 (74.4)	150 (75.0)	150 (74.3)	483 (73.7)	727 (55.4)

NOTE. Data are the no. (%) of subjects, unless otherwise indicated. SD, standard deviation.

[a] Those 18–64 years of age.

[b] Those ⩾65 years of age.

[c] In the past 12 months. The 2008–2009 seasonal influenza vaccine contained A/Brisbane/59/2007 (H1N1)-like virus.

Table 2. Immune Responses after the First Dose of the H1N1 Vaccine

Immunogenicity end point	Placebo				7.50-μg dose			
	Young adults[a] (*n* = 48)	Younger elderly adults[b] (*n* = 38)	Very elderly adults[c] (*n* = 11)	All (*n* = 97)	Young adults[a] (*n* = 200)	Younger elderly adults[b] (*n* = 144)	Very elderly adults[c] (*n* = 57)	All (*n* = 401)
At baseline								
GMT (95% CI)	12.97 (9.3–18.1)	16.2 (11.3–23.1)	29.8 (10.9–81.7)	15.54 (12.2–19.7)	16.8 (14.1–20)	38.8 (23.2–35.8)	51.4 (36.8–71.9)	23.91 (21–27.2)
HI titer ⩾1:40, no. of subjects	10	10	5	25	70	68	40	178
% of subjects (95% CI)	20.8 (10.5–35)	36.3 (15–42)	45.5 (21.3–72)	25.8 (17.4–35.7)	35.0 (28.4–42.0)	47.2 (39.2–55.3)	70.2 (57.3–80.5)	44.4 (39.5–49.4)
After first vaccination								
GMT (95% CI)	12.66 (9.1–17.6)	17.6 (12.2–25.5)	28.6 (10.4–78.8)	15.8 (12.4–20.1)	326.35 (275.9–386)	155.4 (123.4–195.8)	243.9 (167.1–356)	239.9 (210.1–273.9)
HI titer ⩾1:40								
No. of subjects	11	14	5	30	194	130	55	379
% of subjects (95% CI)	22.9 (12.0–37.3)	36.8 (23.4–52.7)	45.5 (21.3–72)	30.9 (21.9–41.1)	97.0 (93.6–98.9)	90.3 (84.3–94.1)	96.5 (88.1–99)	94.5 (91.8–96.5)
Seroconversion								
No. of subjects	0	1	0	1	164	75	28	267
% of subjects (95% CI)	0 (0.0–7.4)	2.6 (0.5–13.5)		1 (0–5.6)	82.0 (76.0–87.1)	52.1 (44–60.1)	49.1 (36.6–61.7)	66.6 (61.7–71.2)
Fold increase in GMT (95% CI)	0.98 (0.9–1.1)	1.1 (0.9–1.3)	1 (0.9–1)	1.02 (1–1.1)	19.43 (15.5–24.4)	5.4 (4.2–6.9)	4.7 (3.1–7.2)	10.03 (8.48–11.87)

NOTE. CI, confidence interval; GMT, geometric mean titer; HI, hemagglutination inhibition.

[a] Those 18–64 years of age.

[b] Those 65–74 years of age.

[c] Those ⩾75 years of age.

⩾18 years old. The study included 2 phases: the active study period, which concluded in November 2009, and the follow-up period, which concluded in April 2010. This report details the immunogenicity and safety results collected during the active study period.

Participants were randomly assigned to receive either placebo or H1N1 vaccine containing 7.5, 15, or 30 μg of HA in a 1:4:4:4 allocation ratio, as stratified by age (<65 years and ⩾65 years). Participants and investigators remained blinded to study group assignments until all participants completed the active study period.

The study was approved by the Johns Hopkins Bloomberg School of Public Health institutional review board and the Aspire institutional review board (San Diego, California). The study was conducted in accordance with the principles of the Declaration of Helsinki and the Standards of Good Clinical Practice (as defined by the International Conference on Harmonisation). All participants provided written, informed consent. All authors contributed to the content of the manuscript, had full access to study data, and vouch for the completeness and accuracy of the data.

Primary and secondary end points. Primary end points were based on US Food and Drug Administration industry guidance for the licensure of pandemic vaccines [22]. Coprimary end points were the percentage of participants achieving an HI antibody titer ⩾1:40 and the seroconversion rate. Seroconversion was defined as a postvaccination HI antibody titer ⩾1:40 (if the baseline HI antibody titer was <1:10) or a ⩾4-fold increase in the postvaccination HI antibody titer (if the HI antibody titer at baseline was ⩾1:10). Secondary end points were the frequency, duration, and intensity of solicited adverse events during the 7 days after vaccination and the incidence of serious adverse events, adverse events of special interest, and new onsets of chronic illness during the study period.

Vaccine. The H1N1 monovalent, unadjuvanted, inactivated, split-virus vaccine was produced by CSL Limited [3]. The 15- and 30-μg doses were supplied in multidose vials of 60 μg of HA per milliliter with thimerosal 0.01% (wt/vol). The 7.5-μg doses were supplied in prefilled syringes that contained 7.5 μg of HA in 0.25 mL of thimerosal-free diluent. Placebo was supplied in multidose vials containing vaccine diluent and thimerosal 0.01% (wt/vol).

Participants and study procedures. Healthy nonpregnant adults ⩾18 years of age were eligible for enrollment. We excluded participants who had a history of an influenza-like illness since April 2009 and those who had received the 2009–2010 seasonal influenza vaccine during the 7 days before the first vaccination.

Table 2. ***(Continued.)***

15-μg dose				30-μg dose			
Young adults[a] (*n* = 196)	Younger elderly adults[b] (*n* = 150)	Very elderly adults[c] (*n* = 48)	All (*n* = 394)	Young adults[a] (*n* = 200)	Younger elderly adults[b] (*n* = 146)	Very elderly adults[c] (*n* = 54)	All (*n* = 399)
17.8 (14.9–21–3)	16.8 (22.2–32.4)	45.1 (32–63.6)	23.31 (20.6–26.4)	12.24 (10.5–14.2)	23.9 (19.8–28.8)	33.1 (23.5–46.7)	17.88 (15.9–20.1)
65	72	35	172	41	60	27	128
33.2 (26.6–40.2)	48 (40.2–55.9)	72.9 (59–83.4)	43.7 (38.7–48.7)	20.5 (15.1–26.8)	41.1 (33.4–49.2)	50 (37.1–62.9)	32.0 (27.5–36.8)
379.65 (314–459)	158 (128.3–194.7)	203.5 (138.7–298.6)	252.06 (219.8–289.1)	502.13 (425.4–592.8)	201.3 (164.4–246.4)	199.9 (138.3–288.9)	317.61 (279.3–361.2)
189	135	45	369	199	139	49	387
96.4 (92.8–98.6)	90 (84.2–93.8)	93.8 (83.2–97.9)	93.7 (90.8–95.9)	99.5 (97.2–100)	95.2 (90.4–97.7)	90.7 (80.1–96)	96.8 (94.5–98.3)
173	93	24	290	189	111	35	335
88.3 (82.9–92.4)	62 (54–69.4)	50 (36.4–63.6)	73.6 (69.0–77.9)	94.5 (90.4–97.2)	76 (68.5–82.2)	64.8 (51.5–76.2)	83.8 (79.8–87.2)
21.33 (17.2–26.5)	5.9 (4.8–7.2)	4.5 (3.2–6.3)	10.81 (9.3–12.6)	41.02 (33.5–50.2)	8.4 (6.9–10.3)	6 (4.2–8.6)	17.76 (15.2–20.8)

Participants received 2 intramuscular vaccinations 21 days apart. Because the injection volume and vaccine presentation differed between treatments, personnel who prepared and administered the study vaccine and placebo had no further involvement in the study.

Safety assessments. We collected reports of local and systemic solicited adverse events with the use of a 7-day diary. Unsolicited adverse events were collected using a 21-day diary. All solicited local adverse events were considered to be related to H1N1 vaccine. The investigator determined the causality of solicited systemic and unsolicited adverse events. Participants used a standard scale to grade the intensity of adverse events. Data regarding new onsets of chronic illness and specified adverse events of special interest, including several neurological (eg, Guillain-Barré syndrome), immune system, and other disorders were collected prospectively. Adverse events of special interest or serious adverse events were to be reported by investigators within 24 h of notification.

A data and safety monitoring board supervised the conduct of the study. Stopping rules were in place for 7 days after each vaccination.

Assessment of influenza-like illness. Participants who reported an influenza-like illness during the active study period were asked to provide nasal and throat swabs for virologic testing. Influenza-like illness was defined as an oral temperature ⩾100.4°F or a history of fever or chills and at least one influenza-like symptom (sore throat, cough, myalgia, headache, diarrhea, vomiting, or malaise).

Laboratory assays. Serum samples were obtained at baseline and 21 days after each vaccination and were tested for HI antibodies to 2009 H1N1, as described elsewhere [3]. Virologic testing of nasal and throat swabs was performed using real-time reverse-transcriptase polymerase chain reaction [3]. Assays were performed by Focus Diagnostics.

Statistical analysis. The sample size for each cohort (200 participants per vaccine antigen dose group and 50 participants per placebo group) was chosen to provide sufficient power to assess the primary immunogenicity end points. The primary end point analyses for each cohort were descriptive and comprised a comparison of the lower confidence bounds of each end point for each treatment group against the US Food and Drug Administration–specified criteria [22]. The 95% confidence intervals (CIs) are provided for descriptive statistics. Proportions were compared using Fisher's exact test when there were 2 groups, and likelihood ratio χ^2 tests were otherwise used. Geometric mean titers (GMTs) were compared on log-transformed titers with the use of Student's *t* test, when there were 2 groups and analysis of variance, or general linear models, when there were >2 groups. Confidence intervals for GMTs were based on log-transformed titers, assuming log-normal distributions.

We performed post hoc exploratory analyses of the effect of

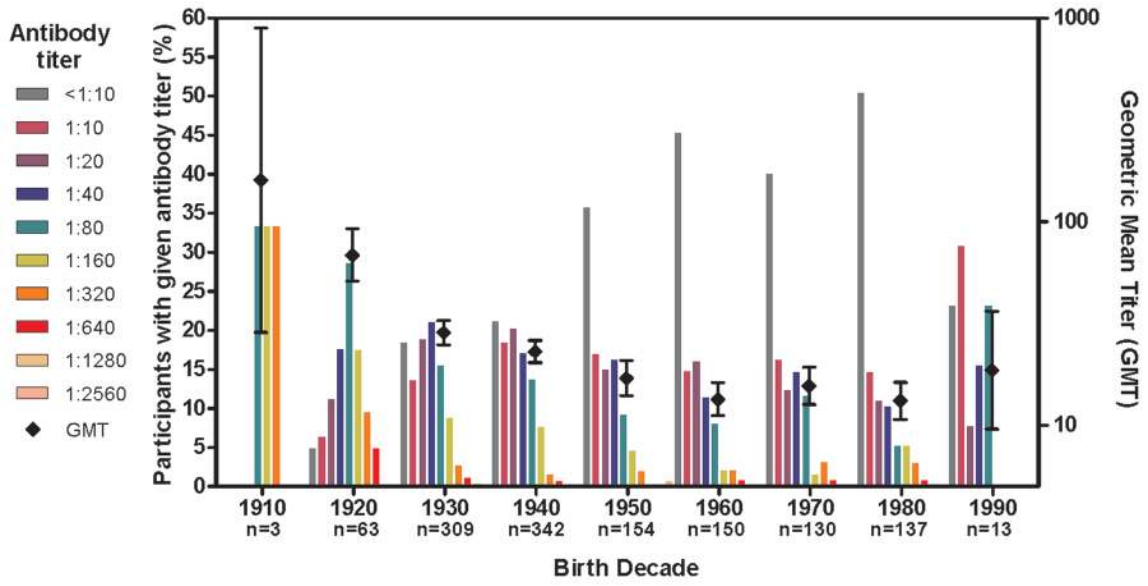

Figure 2. Hemagglutination inhibition (HI) antibody titers against 2009 H1N1 at baseline, by birth decade (1910–1990). The proportion of individuals with given HI antibody titers before immunization is plotted on the left ordinate by birth decade. The geometric mean titers of HI antibody are plotted along the right ordinate by birth decade and are denoted by black circles. *n*, the number of participants born in each decade.

age on antibody response to H1N1 vaccine by further stratifying the older adult cohort into 2 groups: younger elderly adults (age, 65–74 years) and very elderly adults (age, ⩾75 years). We analyzed immunogenicity at baseline and after vaccination, using covariate-adjusted multiple linear regression models and logistic regression models, and calculated GMT ratios to compare different levels of the covariates of interest. Covariates included baseline log HI titers, age group, race, sex, receipt of seasonal influenza vaccine within the past 12 months, and 2-way interaction terms (age group and sex; age group and receipt of seasonal influenza vaccine in the past 12 months). In the regression models, age was classified using the terms "younger adults," "younger elderly adults," and "very elderly adults." Because the numbers of participants of other races were limited, we only compared the responses of black and white participants. Tukey's honestly significant difference test was used for post hoc multiple comparisons involving >2 groups in the multiple linear regression models, including comparisons between antigen dose levels and between the 3 age groups. The data were explored to assess effect modification by antigen dose and characteristics at baseline, as well as the effect of sex and age on the immune response.

RESULTS

Demographic characteristics and baseline clinical characteristics. Between 24 August and 10 September 2009, a total of 1313 participants were enrolled, stratified by age, and randomized to receive placebo or 7.5, 15, or 30 μg of H1N1 vaccine (Figure 1 and Table 1). A total of 658 participants were enrolled in the younger adult cohort, and 655 were enrolled in the older adult cohort. All 1313 participants received the first dose of vaccine or placebo. After the first immunization, 1312 participants provided data for the safety analysis, and 1292 participants provided data for the immunogenicity analysis. The second immunization was received by 1295 participants, of whom 1280 provided data for the immunogenicity analyses (Figure 1). Substantially more older participants (73.7%) than younger participants (37.1%) reported receiving seasonal influenza vaccine in the previous 12 months ($P < .001$, by Fisher's exact test) (Table 1).

Immunogenicity titers of HI antibody at baseline. Before immunization, HI titers ⩾1:40 were observed in 38.9% of participants, more often in older adults (317 [48.9%] of 648 participants) than in younger adults (186 [28.8%] of 644 participants) ($P < .001$, by Fisher's exact test) (Table 2 and Figure 2). Further stratification of the older adult cohort into younger elderly adults and very elderly adults showed that the very elderly adults had higher titers at baseline than did the younger elderly adults: 62.9% of those ⩾75 years of age had baseline titers ⩾1:40, compared with 43.9% of those 65–74 years of age ($P < .001$, by likelihood ratio χ^2 test); GMTs were 41.6 and 25.4, respectively (compared with 15.2 for younger adults) ($P < .001$, by Tukey's honestly significant difference test).

The relationship between age and antibody titer at baseline is shown in detail in Figure 2. Antibody titers were greatest in the 66 very elderly participants born between 1910 and 1930,

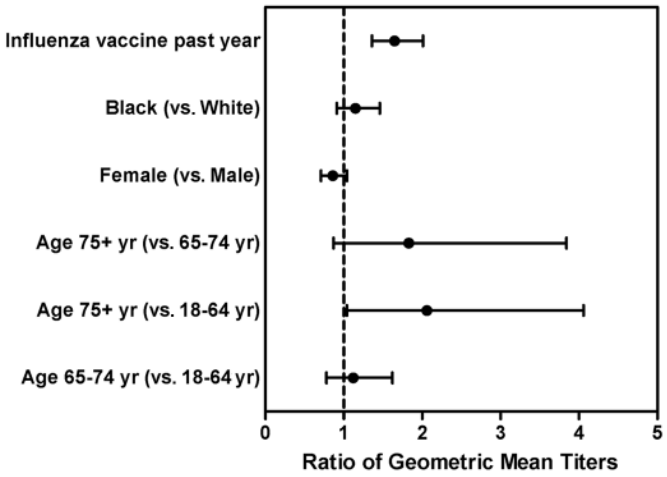

Figure 3. Multivariate analysis of characteristics at baseline and geometric mean titers of hemagglutination inhibition antibody at baseline. Hemagglutination inhibition antibody titers at baseline were analyzed using covariate-adjusted multiple linear regression models with the parameter estimates of the covariates of interest presented as geometric mean titer ratios. Covariates included age, race (black or white), sex, and receipt of seasonal influenza vaccine in the preceding 12 months.

of whom 49 (74%) had titers ⩾1:40 and 20 (30%) had titers ⩾1:160. In contrast, only 77 (13%) of 584 participants born after 1950 had titers ⩾1:40, and 36 (6%) had titers ⩾1:160.

Receipt of seasonal influenza vaccine in the preceding 12 months was associated with higher antibody titers at baseline: 355 (49.6%) of 716 participants who received the 2008–2009 vaccine had baseline HI titers ⩾1:40, compared with 148 (25.7%) of 576 participants who had not received the vaccine ($P < .001$, by Fisher's exact test). Because elderly individuals were more likely to have received seasonal influenza vaccine in the past 12 months than were younger individuals, relative contributions of age and vaccination status were assessed by multiple logistic regression analysis. Although the odds of having a baseline titer ⩾1:40 for older versus younger age groups were reduced after adjustment for previous vaccination status, they remained statistically significant ($P < .001$, by likelihood ratio χ^2 test in 2-way logistic regression). Thus, age ⩾75 years and previous receipt of seasonal influenza vaccine each contributed independently to the antibody titer at baseline (Figure 3).

Vaccine response. After a single vaccination, 7.5, 15, and 30 μg of H1N1 vaccine all produced robust antibody responses in the majority of participants in both age cohorts. A single vaccination with any antigen dose induced HI titers ⩾1:40 in 95% of participants (95% CI, 93.6%–96.1%); placebo recipients did not develop titers greater than levels noted at baseline (Table 2 and Figure 4). Even in subjects ⩾65 years of age, a single H1N1 2009 vaccination was immunogenic at all doses tested, with 92.3% (95% CI, 89.9%–94.2%) achieving HI titers ⩾1:40 and a GMT of 181.4 (95% CI, 163–201.8). Second vaccinations resulted in only minimal increases in antibody titers and seroconversion rates in all dose groups.

Of note, a single 7.5-μg dose of H1N1 vaccine induced HI titers ⩾1:40 in 94.5% of participants (95% CI, 91.8%–96.3%) (Table 2 and Figure 4). The GMT achieved for all recipients of 7.5 μg of HA was 239.9 (95% CI, 210.1–273.9), a 10.3-fold increase in titer (95% CI, 8.48–11.87). Seroconversion after a single 7.5-μg dose was achieved in 66.6% of all participants (95% CI, 61.7%–71.2%) and in 51.2% (95% CI, 44.1%–58.3%) of those ⩾65 years of age. Although the 15-μg and 30-μg HA doses were also immunogenic (Table 2 and Figure 4), significant differences in antibody response were not observed between recipients of the 7.5-μg and 15-μg doses.

A single 7.5-μg dose of this vaccine was also immunogenic in elderly subjects and induced HI titers ⩾1:40 in 92% of elderly participants, with a seroconversion rate of 51.2% (95% CI, 44.1%–58.3%) in those ⩾65 years of age. This vaccine dose was even immunogenic in the very elderly adults, in whom antibody responses were better than expected. Notably, antibody responses in very elderly adults were similar to those in younger elderly adults: 96.5% (95% CI, 88.1%–99.0%) of very elderly adults vs. 90.3% (95% CI, 84.3%–94.1%) of younger elderly adults achieved HI titers ⩾1:40. Similarly, the GMTs achieved were 243.9 (95% CI, 167.1–356.0) in very elderly adults and 155.4 (95% CI, 123.4–195.8) in younger elderly adults. In very elderly adults, an increasing antigen dose did not result in a higher GMT or a higher percentage with HI titers ⩾1:40 (Table 2).

The effects of race and sex on vaccine response were explored using multiple regression analysis. Although there were differ-

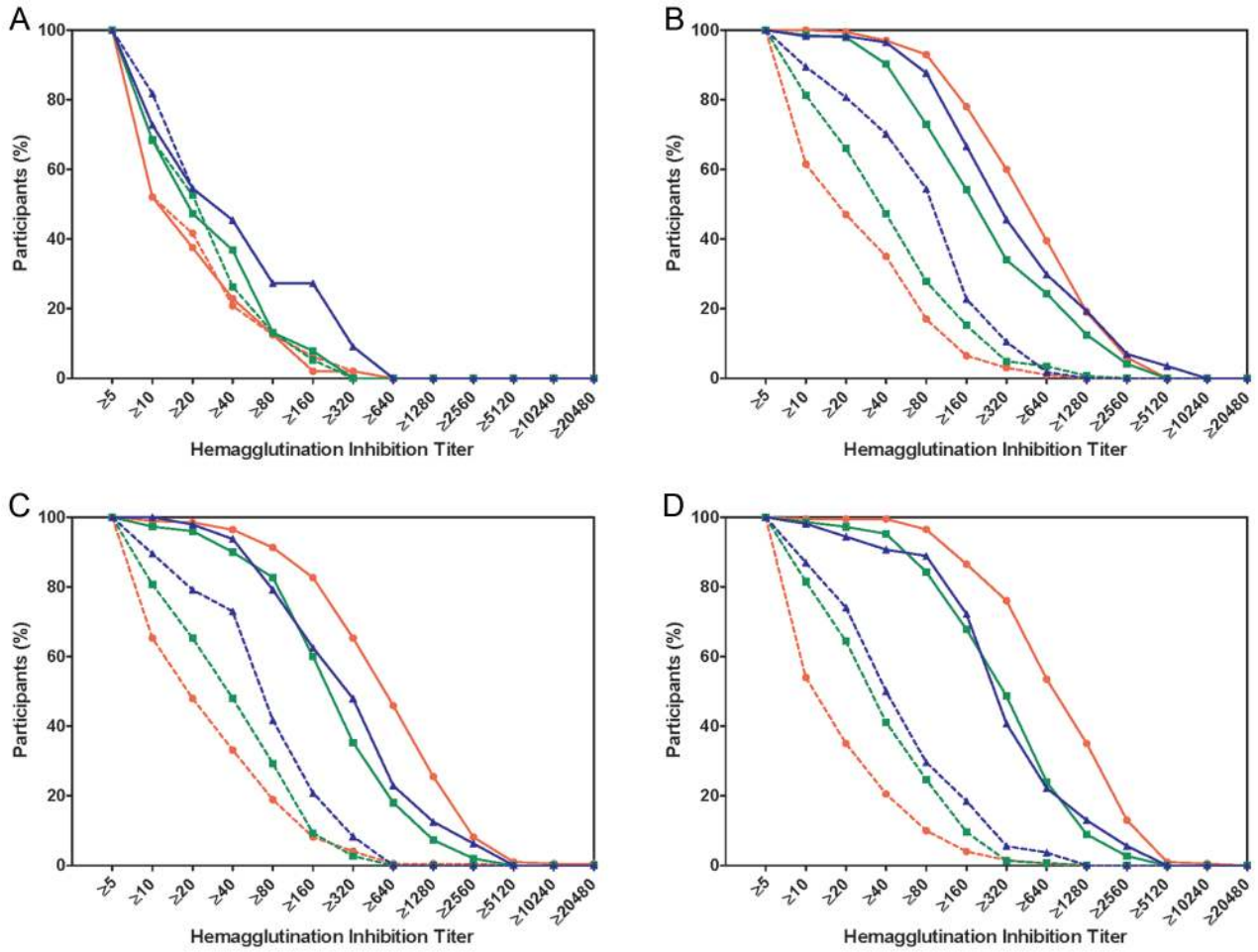

Figure 4. Reverse cumulative distribution of hemagglutination inhibition antibody titers before and after the first dose of 2009 H1N1 vaccine or placebo. *A*, Placebo. *B*, 7.5 μg of hemagglutinin antigen (HA) antigen. *C*, 15 μg of HA antigen. *D*, 30 μg of HA antigen. Red circles denote adults 18–64 years of age; green squares, adults 64–74 years of age; blue triangles, ⩾75 years of age; dashed lines, before first immunization; and solid lines, after first immunization.

ences in baseline and postvaccination immune responses by race, these differences were not evident after adjusting for age. In general, sex was not associated with immune response. However, there was a sex–age group interaction ($P < .003$), with women ⩾75 years of age having significantly higher postvaccination titers than men in the same age group (Tukey's honestly significant difference on multivariate model parameters, $P = .014$) (GMT, 273.2 vs. 128.5) and men 65–74 years of age (Tukey's honestly significant difference, $P < .006$) (GMT, 273.2 vs. 159.6).

Safety. No significant safety concerns were identified. There were no deaths, serious adverse events, adverse events of special interest, or new onsets of chronic illness that were assessed as related to the H1N1 vaccine. Fifteen unrelated serious adverse events occurred, including an unexpected death of a 41-year-old woman from an overdose with opioids, benzodiazepines, and stimulants. This was the only adverse event that resulted in participant withdrawal. Stopping rules were triggered once because of the occurrence of serious adverse events, but enrollment resumed when the events were assessed as unrelated to study vaccine by the investigators.

The H1N1 vaccine was well tolerated; 85.6% of solicited adverse events were of mild intensity (Figure 5). The most frequently reported solicited local adverse events were tenderness and pain at the injection site. The most frequently reported solicited systemic adverse events were headache, myalgia, and malaise. Solicited adverse events were generally reported more frequently with the higher antigen doses, compared with the lower doses and placebo, and after the first vaccination compared with after the second vaccination (Figure 5). A higher

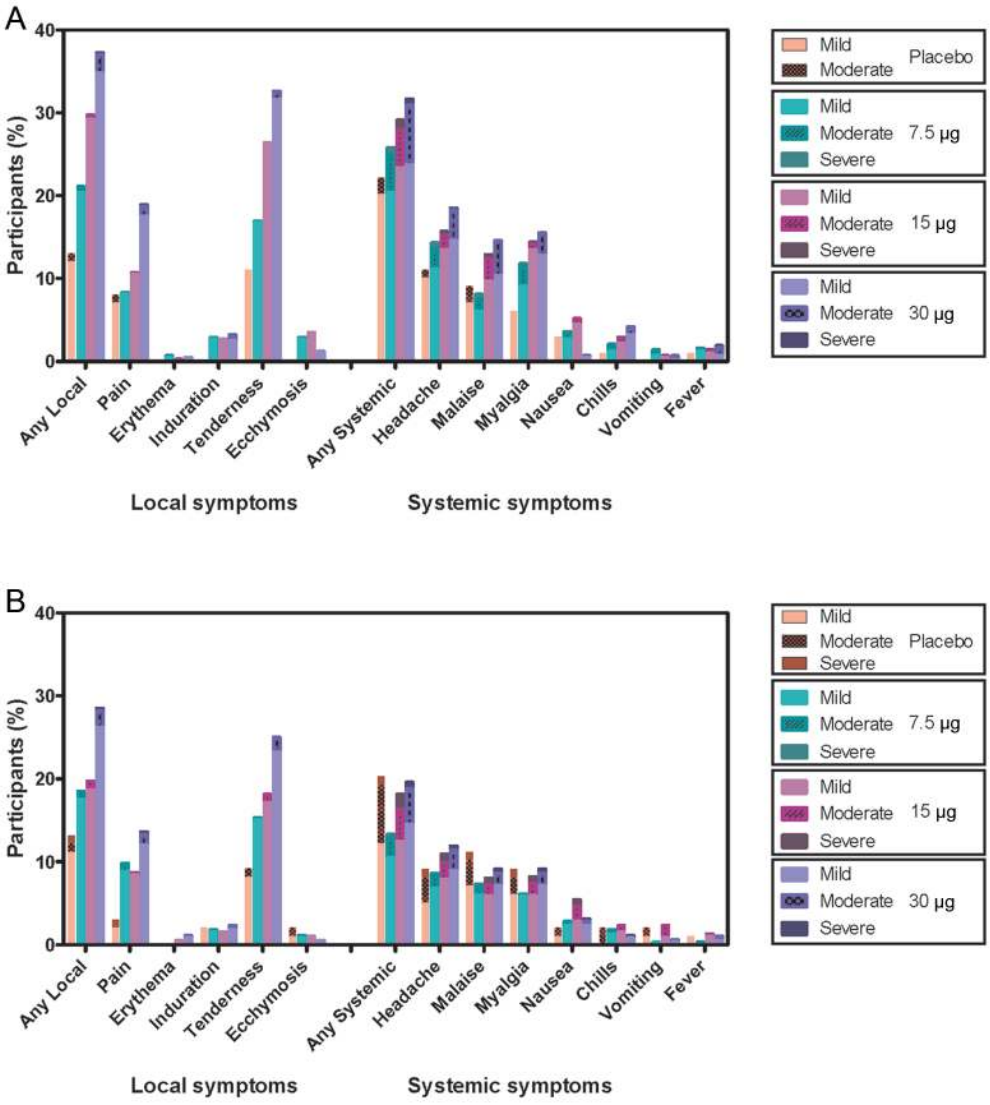

Figure 5. Solicited reports of adverse events 7 days after the first dose (*A*) and second dose (*B*) of the 2009 H1N1 vaccine, The severity of local and systemic adverse events is shown by dosage. Red denotes placebo; green, 7.5 μg of hemagglutinin antigen (HA); purple, 15 μg of HA; and blue, 30 μg of HA.

proportion of younger adults reported solicited adverse events (45.7% of younger adults and 24.9% of older adults reported local adverse events).

Unsolicited adverse events were reported by 26.9% of participants. The most frequently reported events were headache, oropharyngeal pain, and cough. The majority (89.4%) of events were mild or moderate in intensity.

Of the 26 participants who reported an influenza-like illness during the active study period, 2 had laboratory-confirmed 2009 H1N1 infection.

DISCUSSION

This unadjuvanted 2009 H1N1 vaccine was well tolerated and induced high levels of HI antibody in all age groups, even after a single dose of 7.5 μg of HA. This dose, which was one-half the per-strain dose used in inactivated seasonal influenza vaccines, met and exceeded the US Food and Drug Administration immunogenicity criteria for influenza vaccines for both healthy younger and elderly adults [22]. For young and elderly adults, the GMTs achieved were 326 and 176, respectively, which is well above the levels presumed to confer protective immunity.

The large cohorts of young and older individuals enrolled in this study allowed us to assess levels of preexisting H1-subtype HI antibody by decade of life. The patterns identified in our study are consistent with previous serosurveys that assessed levels of H1N1 neutralizing antibody [18, 21]. We found that individuals born before 1930 had substantially higher HI GMTs at baseline than did those born after 1930 and that a

high proportion of those born after 1950 were H1N1 seronegative (HI titer, <1:10) (Figure 2 and Table 1). Because the 2009 H1N1 HA is antigenically most similar to H1N1 viruses that circulated shortly after 1918, these data are also consistent with the antigenic evolution of H1, which underwent drift events in 1928–1929 and 1934–1936 and intrasubtypic reassortment in 1947–1948 [19]. This finding is also consistent with the age distribution of infection with the 2009 H1N1 virus, in which the lowest attack rates are seen in individuals >60 years of age [13], and with experimental data indicating that vaccination with a 1918-like virus protects mice against lethal challenge with 2009 H1N1 [23].

More than 90% of participants, including very elderly subjects, achieved an HI titer of ⩾1:40 after 1 dose of vaccine. The antibody titers achieved in this population met or exceeded those achieved in the younger elderly population (Table 2 and Figure 4). It is possible that the antibody responses in very elderly adults reflect the combined effects of antigenic priming and immunosenescence relative to younger adults. In addition, in elderly individuals, the antibody responses to the 2009 H1N1 vaccine were distinctly different from responses previously observed after administration of seasonal influenza vaccine. The meta-analysis performed by Goodwin et al [14] showed that participants ⩾65 years of age were approximately one-half as likely as younger participants to develop HI antibody titers ⩾1:40 to the H1N1 component of the trivalent seasonal influenza vaccines [14], which are formulated to contain 15 μg of H1 HA. In contrast, we observed that 92% of older participants and 97% of younger participants who received 7.5 μg of 2009 H1N1 HA developed HI titers ⩾1:40. The reasons for the robust antibody responses to the 2009 H1N1 HA observed in young and older adults alike are unknown, but they are consistent with previous studies [3, 8, 9], and they could reflect priming of conserved B cell or T cell epitopes [24] via previous infection with antigenically distinct influenza strains.

Suboptimal antibody responses to seasonal influenza vaccines are frequently observed in elderly individuals [14]; as a consequence, strategies to enhance immunogenicity have included the use of adjuvants [25] and increasing the antigen content of vaccine [17, 25, 26]. A trivalent influenza vaccine containing MF-59 adjuvant (FluAd; Novartis) has been licensed in Europe for use in elderly individuals for several years, and an unadjuvanted trivalent influenza vaccine containing 60 μg of each influenza HA (Fluzone High-Dose; Sanofi) has recently been licensed by the US Food and Drug Administration [27]. In contrast, our data show that a single 7.5-μg dose of unadjuvanted 2009 H1N1 HA is highly immunogenic in elderly individuals and that antibody responses were not enhanced by administration of 15 or 30 μg of HA. Additional research is needed to elucidate the mechanisms (eg, conserved epitopes, antineuraminidase antibodies, and T cell–mediated immunity) underlying the enhanced immunogenicity of the 2009 H1N1 vaccine in all populations, especially elderly adults.

Our study also afforded opportunities to perform exploratory post hoc analyses of the effect of sex and black and white race on antibody responses. A previous study showed that the HI antibody response achieved in women who received one-half of a dose of seasonal influenza vaccine was comparable to the response achieved in men who receive a full dose [28], and interest in this issue increased with the onset of the pandemic and the potential need for dose-sparing [29]. With the exception of a difference in the fold increase in the GMT achieved in women ⩾75 years of age, we saw no difference in antibody responses by sex. However, the high antibody levels produced in response to this vaccine at the lowest dose tested may have masked potential sex differences. Assessment of variations in immune response by race and ethnic group is particularly important for pandemic vaccines where immediate global use is intended, especially given recent data suggesting that there may be racial and ethnic disparities in the severity of H1N1 disease [30]. The composition of our trial population meant that we could only assess responses in black and white participants. Although it was reassuring that no differences were detected, further assessment is needed to evaluate the effects in larger, more-diverse populations.

In conclusion, we confirmed that a single 7.5-μg dose of an unadjuvanted 2009 H1N1 vaccine is highly immunogenic in healthy younger adults, and we demonstrated that this vaccine dose is also immunogenic in younger elderly and very elderly individuals. The immunogenicity of the 7.5-μg dose may have immediate policy implications in instances where dose-sparing strategies for use of the monovalent vaccine are necessary [9]. The immunogenicity of 2009 H1N1 HA for elderly individuals has important future implications. 2009 H1N1 influenza is a component of the 2010 Southern Hemisphere and the 2010–2011 Northern Hemisphere trivalent seasonal influenza vaccines. Therefore, the immunogenicity of 2009 H1N1 HA for elderly individuals will become increasingly relevant as the elderly population once again become a primary target population for influenza immunization.

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