The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 17, 2009 VOL. 361 NO. 25

Response to a Monovalent 2009 Influenza A (H1N1) Vaccine

Michael E. Greenberg, M.D., M.P.H., Michael H. Lai, B.Med.Sc., M.B., B.S., M.Med.Sc., Gunter F. Hartel, M.S., Ph.D., Christine H. Wichems, Ph.D., Charmaine Gittleson, B.Sc., M.B., B.Ch., Jillian Bennet, M.Sc., M.P.H., Gail Dawson, B.Pharm., Wilson Hu, M.D., M.B.A., Connie Leggio, B.Sc., Diane Washington, M.D., and Russell L. Basser, M.B., B.S., M.D., F.R.A.C.P.

ABSTRACT

BACKGROUND

A novel 2009 influenza A (H1N1) virus is responsible for the first influenza pandemic in 41 years. A safe and effective vaccine is needed. A randomized, observer-blind, parallel-group trial evaluating two doses of an inactivated, split-virus 2009 H1N1 vaccine in healthy adults between the ages of 18 and 64 years is ongoing at a single site in Australia.

METHODS

We evaluated the immunogenicity and safety of the vaccine after each of two scheduled doses, administered 21 days apart. A total of 240 subjects, equally divided into two age groups (<50 years and ≥50 years), were enrolled and underwent randomization to receive either 15 μg or 30 μg of hemagglutinin antigen by intramuscular injection. We measured antibody titers using hemagglutination-inhibition and microneutralization assays at baseline and 21 days after vaccination. The coprimary immunogenicity end points were the proportion of subjects with antibody titers of 1:40 or more on hemagglutination-inhibition assay, the proportion of subjects with either seroconversion or a significant increase in antibody titer, and the factor increase in the geometric mean titer.

RESULTS

By day 21 after the first dose, antibody titers of 1:40 or more were observed in 114 of 120 subjects (95.0%) who received the 15-μg dose and in 106 of 119 subjects (89.1%) who received the 30-μg dose. A similar result was observed after the second dose of vaccine. No deaths, serious adverse events, or adverse events of special interest were reported. Local discomfort (e.g., injection-site tenderness or pain) was reported by 56.3% of subjects, and systemic symptoms (e.g., headache) by 53.8% of subjects after each dose. Nearly all events were mild to moderate in intensity.

CONCLUSIONS

A single 15-μg dose of 2009 H1N1 vaccine was immunogenic in adults, with mild-to-moderate vaccine-associated reactions. (ClinicalTrials.gov number, NCT00938639.)

From Clinical Research and Development, CSL, Parkville, VIC, Australia. Address reprint requests to Dr. Greenberg at Clinical Research and Development, CSL, 45 Poplar Rd., Parkville, VIC 3052, Australia, or at michael.greenberg@csl.com.au.

A preliminary version of this article (10.1056/NEJMoa0907413) was published on September 10, 2009, at NEJM.org.

N Engl J Med 2009;361:2405-13.

The rapid global spread of a novel 2009 influenza A (H1N1) virus (2009 H1N1) prompted the World Health Organization (WHO), on June 11, 2009, to declare the first influenza pandemic in 41 years.[1] In the Southern Hemisphere, 2009 H1N1 infection has been dominant during the current influenza season.[2] In the Northern Hemisphere, the incidence of 2009 H1N1 infection has increased substantially during the early part of the influenza season. The availability of safe and effective vaccines is a critical component of efforts to prevent 2009 H1N1 infection and mitigate the overall effect of the pandemic.[3,4]

Shortly after the identification of 2009 H1N1, influenza vaccine manufacturers, in conjunction with public health and regulatory agencies, started developing a 2009 H1N1 vaccine.[5] The sense of urgency was particularly notable in the Southern Hemisphere, where the timing of the pandemic coincided with the onset of winter. Ideally, clinical trials are needed to establish the safety and adverse-effect profiles of the new vaccines and to confirm the optimal dose and regimen.[6]

We undertook a clinical trial in healthy adults to examine the immunogenicity, safety, and tolerability of two different doses of a monovalent, split-virus 2009 H1N1 influenza vaccine (H1N1 vaccine). The vaccine was manufactured with the same procedures that have been used for the production of the company's seasonal trivalent inactivated vaccine. We examined a two-dose regimen of either 15 μg or 30 μg of hemagglutinin antigen, because there was uncertainty as to whether a higher antigen content or a two-dose series might be required to produce a satisfactory immune response. We enrolled equal numbers of subjects 50 years of age or older and below the age of 50 years to explore potential age-related differences in immune response that might result from previous exposure to H1N1 viruses that were displaced from circulation by the H2N2 subtype in the 1957–1958 influenza pandemic.[7]

In the current pandemic, rapid sharing of clinical-trial findings is critical, since such data may assist in the planning of national vaccination programs. Earlier, we presented results in a preliminary report (available at NEJM.org) from our ongoing Australian study in healthy adults after the first of two scheduled vaccinations. This report includes results that are available to date after the second vaccination.

Methods

Study Design

This phase 2, prospective, randomized, observer-blind, parallel-group clinical trial was conducted at a single site in Adelaide, Australia (CMAX, a division of the Institute of Drug Technology). The purpose of this study was to evaluate the immunogenicity and safety of two different doses of the H1N1 vaccine in healthy adults between the ages of 18 and 64 years in a two-dose regimen. All subjects provided written informed consent.

The randomization code was prepared by a statistician, employed by CSL Limited, with the use of SAS software (version 9.1.3) and JMP (version 8.0.1) (SAS Institute); permuted-block randomization was used. The randomization code was provided to the vaccine administrator, who was aware of study-group assignments, as a list in a sealed envelope, although all subjects and investigators were unaware of such assignments.

The study was approved by the Bellberry Human Research Ethics Committee (Adelaide, Australia) and was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonization), and Australian regulatory requirements. All authors contributed to the content of the manuscript, had full access to all study data, and vouch for the completeness and accuracy of the data.

Vaccine

The H1N1 vaccine, a monovalent, unadjuvanted, inactivated, split-virus vaccine, was produced by CSL Biotherapies (Parkville, Australia). The seed virus was prepared from the reassortant vaccine virus NYMC X-179A (New York Medical College, New York), derived from the A/California/7/2009 (H1N1) virus, one of the candidate reassortant vaccine viruses recommended by the WHO.[8,9] The vaccine was prepared in embryonated chicken eggs with the same standard techniques that are used for the production of seasonal trivalent inactivated vaccine[10] and was presented in 10-ml multidose vials with thimerosal added as a preservative (final concentration, 0.01% weight per volume). The two doses were 15 μg of hemagglutinin antigen per 0.25-ml dose and 30 μg of hemagglutinin antigen per 0.5-ml dose.

Subjects and Study Procedures

Healthy, nonpregnant adults between the ages of 18 and 64 years were eligible for enrollment. We excluded subjects with confirmed or suspected 2009 H1N1 infection and those who had received an experimental influenza vaccine during the preceding 6 months.

A total of 240 eligible subjects underwent randomization to receive either 15 μg or 30 μg of hemagglutinin antigen in a 1:1 ratio. An equal number of subjects from 18 to 49 years of age and from 50 to 64 years were included. Subjects received two doses of the assigned vaccine, administered 21 days apart. Each dose was administered intramuscularly into the deltoid muscle. Since the injection volume differed between the two study doses, personnel who prepared and administered the study vaccine had no further involvement in the study.

Safety Assessments

We collected solicited reports of local and systemic adverse events, using a 7-day diary card. Unsolicited reports of adverse events were collected in a 21-day diary card. All solicited local adverse events were considered to be related to the H1N1 vaccine, whereas the investigator assessed the causality of solicited systemic and unsolicited adverse events. Subjects used a standard scale to grade adverse events (Tables 1 and 2 in the Supplementary Appendix).

Because of the novelty of the pandemic H1N1 strain, we prospectively collected data relating to the occurrence of select adverse events of special interest. These events included several neurologic (e.g., Guillain–Barré syndrome), immune-system, and other disorders. Any adverse events of special interest or serious adverse event was to be reported within 24 hours.

A safety-review committee monitored the safety of the study. Stopping rules were in place during the 7 days after vaccination but were not met.

Assessment of Influenza-like Illness

Subjects who reported having an influenza-like illness were asked to provide specimens of nasal and throat swabs for virologic testing. An influenza-like illness was defined as an oral temperature of more than 38°C (100.4°F) or a history of fever or chills and at least one influenza-like symptom.

Laboratory Assays

Anti-influenza antibody titers were measured at enrollment and 21 days after each vaccination.

Table 1. Demographic Characteristics of the Subjects.*

Characteristic	15-μg Vaccine Dose (N=120)			30-μg Vaccine Dose (N=120)			All Subjects (N=240)
	18–49 Yr (N=58)	50–64 Yr (N=62)	All Ages (N=120)	18–49 Yr (N=62)	50–64 Yr (N=58)	All Ages (N=120)	
Age — yr							
Mean	31.0±9.7	57.3±4.3	44.6±15.1	29.8±9.7	56.8±3.6	42.9±15.4	43.7±15.3
Median	28	58	50	26	56	48	50
Range	18–49	50–64	18–64	18–49	50–64	18–64	18–64
Sex — no. (%)							
Male	24 (41.4)	29 (46.8)	53 (44.2)	24 (38.7)	29 (50.0)	53 (44.2)	106 (44.2)
Female	34 (58.6)	33 (53.2)	67 (55.8)	38 (61.3)	29 (50.0)	67 (55.8)	134 (55.8)
Race — no. (%)†							
White	50 (86.2)	61 (98.4)	111 (92.5)	54 (87.1)	57 (98.3)	111 (92.5)	222 (92.5)
Other	8 (13.8)	1 (1.6)	9 (7.5)	8 (12.9)	1 (1.7)	9 (7.5)	18 (7.5)
Received 2009 Southern Hemisphere seasonal influenza vaccine — no. (%)‡	25 (43.1)	30 (48.4)	55 (45.8)	24 (38.7)	29 (50.0)	53 (44.2)	108 (45.0)

* Plus–minus values are means ±SD.
† Race was self-reported.
‡ The 2009 Southern Hemisphere seasonal influenza vaccine contained 15 μg of hemagglutinin antigen of each of the following strains: A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Florida/4/2006 (B).

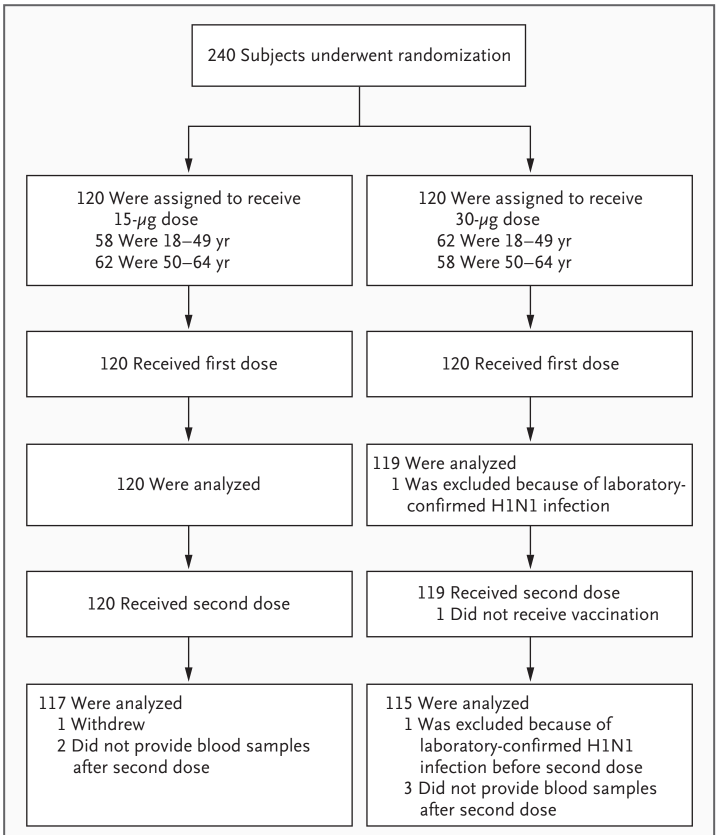

Figure 1. **Enrollment and Outcomes.**

The immunogenicity of the H1N1 vaccine was evaluated with the use of hemagglutination-inhibition and microneutralization assays with methods that have been described previously[11,12] (for details, see the Supplementary Appendix, available with the full text of this article at NEJM.org). Virologic testing of nasal- and throat-swab specimens was performed with the use of the protocol of the Centers for Disease Control and Prevention for real-time reverse-transcriptase–polymerase-chain-reaction assay for 2009 H1N1 virus.[13] All laboratory assays were performed by Focus Diagnostics.

PRIMARY AND SECONDARY END POINTS

The three coprimary immunogenicity end points after vaccination were chosen according to international guidelines used to evaluate influenza vaccines.[14,15] The coprimary immunogenicity end points were the proportion of subjects with antibody titers of 1:40 or more on hemagglutination-inhibition assay, the proportion of subjects with either seroconversion or a significant increase in antibody titer, and the factor increase in the geometric mean titer.

The secondary safety end points were the frequency, duration, and intensity of adverse events after vaccination (solicited events for 7 days and unsolicited events for 21 days) and the incidence of serious adverse events and adverse events of special interest during the study period.

STATISTICAL ANALYSIS

A sample size of 120 subjects per study group was chosen because it provided sufficient power to assess the primary immunogenicity end points. The primary and secondary end-point analyses were descriptive and consisted of an assessment of the lower confidence bounds of each end point for each study group. On the assumption of a population seroconversion rate of 53%, the study had a power of at least 80% with 120 subjects per group to show the seroconversion rate to be significantly more than 40%. For categorical variables, statistical summaries included counts and percentages relative to the appropriate population. The safety population included all subjects who received a dose of H1N1 vaccine. The population that could be evaluated included all subjects in the safety population who provided serum samples at baseline and after vaccination. The 95% confidence intervals, which were calculated on the basis of the binomial distribution, are provided for descriptive statistics.

RESULTS

STUDY SUBJECTS

From July 22 to July 26, 2009, we enrolled 240 subjects, who underwent randomization (Table 1 and Fig. 1). All subjects received a dose of H1N1 vaccine and were included in the safety population. Though all 240 subjects provided a blood sample before and after the first dose of vaccine, 1 subject in the 30-μg dose group tested positive for 2009 H1N1 influenza 8 days after the first vaccination and was excluded from all immunogenicity analyses; thus the immunogenicity analyses after the first vaccination included 239 subjects. Of the 240 subjects, 1 subject declined the second vaccination; thus 239 of the 240 subjects received a second dose of vaccine. Of the 239 subjects who received the second dose, 6 subjects were excluded because they did not provide blood samples, and the subject who tested positive for 2009 H1N1 influenza 8 days after the first dose was also exclud-

Table 2. Immune Responses after the First and Second Dose of the H1N1 Vaccine, as Measured on Hemagglutination-Inhibition (HI) Assay.*

Immunogenicity End Point	15-μg Vaccine Dose			30-μg Vaccine Dose		
	18–49 Yr	50–64 Yr	All Ages	18–49 Yr	50–64 Yr	All Ages
Baseline						
No. of subjects	58	62	120	61	58	119
Subjects with HI titer ≥1:40 — % (95% CI)	32.8 (21.0–46.3)	27.4 (16.9–40.2)	30.0 (22.0–39.0)	32.8 (21.3–46.0)	13.8 (6.1–25.4)	23.5 (16.2–32.2)
Geometric mean titer — value (95% CI)	18.3 (13.0–25.9)	15.0 (11.4–19.6)	16.5 (13.3–20.5)	18.4 (13.1–25.9)	10.9 (8.4–14.3)	14.3 (11.4–17.8)
After first dose						
No. of subjects	58	62	120	61	58	119
Subjects with HI titer ≥1:40 — % (95% CI)	96.6 (88.1–99.6)	93.5 (84.3–98.2)	95.0 (89.4–98.1)	98.4 (91.2–100.0)	79.3 (66.6–88.8)	89.1 (82.0–94.1)
Subjects with seroconversion or significant increase in titer — % (95% CI)	77.6 (64.7–87.5)	71.0 (58.1–81.8)	74.2 (65.4–81.7)	85.2 (73.8–93.0)	77.6 (64.7–87.5)	81.5 (73.4–88.0)
Geometric mean titer — value (95% CI)	277.3 (201.7–381.1)	140.4 (102.5–192.4)	195.1 (155.2–245.3)	474.5 (354.1–635.9)	159.4 (102.8–247.2)	278.8 (211.6–367.4)
Factor increase in geometric mean titer — value (95% CI)	15.1 (10.0–23.0)	9.4 (6.4–13.8)	11.8 (8.9–15.7)	25.8 (17.0–39.1)	14.6 (9.7–22.0)	19.5 (14.6–26.2)
After second dose						
No. of subjects	55	62	117	58	57	115
Subjects with HI titer ≥1:40 — % (95% CI)	98.2 (90.3–100.0)	98.4 (91.3–100.0)	98.3 (94.0–99.8)	100.0 (93.8–100.0)	93.0 (83.0–98.1)	96.5 (91.3–99.0)
Subjects with seroconversion or significant increase in titer — % (95% CI)	83.6 (71.2–92.2)	80.6 (68.6–89.6)	82.1 (73.9–88.5)	87.9 (76.7–95.0)	91.2 (80.7–97.1)	89.6 (82.5–94.5)
Geometric mean titer — value (95% CI)	320.0 (241.0–424.9)	215.6 (165.1–281.5)	259.6 (213.6–315.4)	470.9 (371.9–596.3)	230.4 (159.8–332.3)	330.4 (264.2–413.3)
Factor increase in geometric mean titer — value (95% CI)	18.0 (12.2–26.6)	14.4 (10.2–20.5)	16.0 (12.4–20.7)	26.0 (17.7–38.3)	20.8 (14.4–30.0)	23.3 (17.9–30.3)

* The immunogenicity end points were the proportion of subjects who had an antibody titer of 1:40 or more, the proportion of subjects who had either seroconversion (a prevaccination titer of less than 1:10 with a postvaccination HI antibody titer of 1:40 or more) or an increase by a factor of four or more in antibody titer, and the factor increase in the geometric mean titer.

ed, so 232 subjects were included in the immunogenicity analyses. The single withdrawal from the study was not related to an adverse event. Of the 240 subjects, 45.0% reported having received a 2009 Southern Hemisphere seasonal trivalent inactivated vaccine. The proportion of subjects who received the 2009 seasonal vaccine did not differ between the age groups (P=0.24 by Fisher's exact test).

IMMUNOGENICITY

At baseline, 64 of 239 subjects (26.8%) had antibody titers of 1:40 or more on hemagglutination-inhibition assay (Table 2 and Fig. 2, and Fig. 1 in the Supplementary Appendix). The proportion of subjects with a baseline antibody titer of 1:40 or more was significantly higher in younger subjects than in older subjects (P=0.04 by Fisher's exact test), with no significant difference between the dose groups (P=0.31). Similarly, there were significant differences in baseline geometric mean titers (GMTs) between age groups (P=0.02) but not between dose groups (P=0.35) (Table 2). Baseline titers of 1:40 or more on hemagglutination-inhibition assay were observed in 35 of 108 subjects who had received the 2009 seasonal vaccine (32.4%; 95% confidence interval [CI], 24.3 to 41.7), as compared with 29 of 132 subjects who had not received the seasonal vaccine (22.0%; 95% CI, 15.8 to 29.8; P=0.08 by Fisher's exact test).

A single 15-μg or 30-μg dose of the H1N1 vaccine produced a robust immune response in a

Table 3. Geometric Mean Titers and Factor Increases in the Geometric Mean Titer after the First and Second Dose of the H1N1 Vaccine, as Measured on Microneutralization Assay.

Dose and Age Group	Geometric Mean Titer			Factor Increase in Geometric Mean Titer from Baseline	
	Baseline	After First Dose	After Second Dose	After First Dose	After Second Dose
	value (95% confidence interval)				
15-μg dose	13.6 (10.7–17.4)	338.0 (240.5–475.0)	447.2 (340.6–587.2)	24.8 (17.6–35.1)	32.3 (24.0–43.5)
Age 18–49 yr	17.7 (11.9–26.5)	651.6 (415.1–1022.7)	707.9 (487.3–1028.2)	36.7 (22.2–60.7)	38.0 (23.4–61.6)
Age 50–64 yr	10.6 (8.0–14.1)	183.0 (114.8–291.7)	297.6 (204.9–432.2)	17.2 (10.7–27.6)	28.0 (19.3–40.7)
30-μg dose	13.0 (9.8–17.4)	517.4 (347.9–769.4)	593.5 (433.2–813.2)	39.7 (26.7–59.1)	46.5 (32.8–65.9)
Age 18–49 yr	21.5 (13.5–34.2)	1182.1 (759.5–1840.0)	1163.3 (841.2–1608.8)	54.9 (31.8–95.0)	55.8 (33.5–92.9)
Age 50–64 yr	7.7 (5.7–10.4)	217.0 (118.8–396.4)	299.3 (183.2–488.9)	28.2 (15.8–50.4)	38.6 (23.8–62.7)

majority of subjects (Table 2 and Fig. 2). Postvaccination titers of 1:40 or more on hemagglutination-inhibition assay were observed in 95.0% (95% CI, 89.4 to 98.1) of recipients of the 15-μg dose and in 89.1% (95% CI, 82.0 to 94.1) of the recipients of the 30-μg dose (Table 2 and Fig. 2). Seroconversion or a significant increase in titer on hemagglutination-inhibition assay occurred in 77.8% of subjects, and the effect was similar between the two study groups (Table 2). The immune response that was observed after the first dose of vaccine was sustained after the second dose (Table 2 and Fig. 2).

After a single vaccination, there was a substantial rise in GMTs on hemagglutination-inhibition assay, with a significantly higher factor increase in recipients of the 30-μg dose (P=0.02) (Table 2, and Fig. 2 in the Supplementary Appendix). We also observed age-related differences. Subjects who were 50 years of age or older had a significantly lower factor increase in the GMT than those under the age of 50 years (P=0.01). This age-related effect was reflected in all measures of immunogenicity.

In general, the pattern of antibody responses, as measured by the microneutralization assay, was similar to those observed with the hemagglutination-inhibition assay (Table 3 and Fig. 2, and Fig. 2 in the Supplementary Appendix). Baseline microneutralization GMTs in the younger age group were significantly higher than those in the older age group (P<0.001). Postvaccination microneutralization GMTs were also significantly higher in the younger age group than in the older age group, regardless of dose (P<0.001).

We performed an additional analysis examining the effect of baseline serostatus on the immune response to H1N1 vaccination. Subjects who were seronegative at baseline (with a hemagglutination-inhibition or microneutralization titer of <1:10) had lower GMTs after a single vaccination than those with baseline titers of 1:10 or more (Table 3 in the Supplementary Appendix). However, subjects who were seronegative at baseline had significantly higher factor increases in the GMT (P<0.001 for both hemagglutination-inhibition and microneutralization assays). The proportion of subjects who were seronegative at baseline and who achieved seroconversion after a single vaccination was 87.9% (95% CI, 79.4 to 93.8) on the hemagglutination-inhibition assay and 75.6% (95% CI, 67.4 to 82.5) on the microneutralization assay. Among subjects with a baseline titer of 1:10 or more, the proportion of those achieving seroconversion after the first dose of vaccine was 71.6% (95% CI, 63.6 to 78.7) on the hemagglutination-inhibition assay and 77.9% (95% CI, 68.7 to 85.4) on the microneutralization assay.

ADVERSE EVENTS

No deaths, serious adverse events, or adverse events of special interest were reported. Stopping rules were not triggered, and there were no withdrawals because of adverse events. After the first or second vaccination, at least one solicited local adverse event was reported by 56.3% of subjects, and at least one solicited systemic adverse event was reported by 53.8% of subjects. The most commonly reported solicited local events were injection-site tenderness and pain, and the most commonly reported solicited systemic events were headache, malaise, and myalgia (Fig. 3, and Table 4 in the Supplementary Appendix). The majority of solicited adverse events (86.3%) were mild in intensity. Generally, the pattern and frequency of solicited adverse events after the second vaccination were similar to those observed after the first vaccination (Fig. 3). Notably, the frequency of some solicited adverse events was significantly higher in the 30-μg dose group than in the 15-μg dose group. Unsolicited adverse events after the first or second vaccination were reported by 45.0% of subjects; of these events, 9.2% were considered related to study vaccine (Table 5 in the Supplementary Appendix). The most commonly reported unsolicited events were headache, oropharyngeal pain, and back pain. The majority of events (64.7%) were mild in intensity.

Three subjects had an influenza-like illness, one of whom tested positive for 2009 H1N1 on day 8 after vaccination. The remaining two subjects tested negative for 2009 H1N1.

DISCUSSION

A single 15-μg dose of unadjuvanted 2009 H1N1 vaccine resulted in titers of 1:40 or more on hemagglutination-inhibition assay in 95.0% of adult subjects, despite the prevailing assumption that two doses of vaccine would be required. A second dose of vaccine conferred little additional clinical benefit. These results help to inform pandemic planning, especially in light of widespread concern about vaccine availability because of low manufacturing yields.[16] The high level of immune protection afforded by a single 15-μg dose should improve the coverage and logistics of mass H1N1 vaccination programs.

The robust immune response to the H1N1 vaccine after a single dose was unanticipated. Much of the current global pandemic planning is predicated on previous experience that two doses of vaccine are required to elicit a protective immune response in populations that are immunologically naive to a new influenza strain.[17-21]

The initiation of the study coincided with the peak of the first pandemic wave in Australia. The weekly age-standardized H1N1 notification rate in South Australia, the state in which the study site is located, was higher than the national average at that time (113.6 per 100,000 population in South Australia, and 81.8 per 100,000 population in Australia).[22] However, we do not believe that intercurrent infection significantly contributed to the postvaccination response, since we monitored all subjects for influenza-like illness, and only one subject tested positive for 2009 H1N1 during the 21 days after the first vaccination.

The proportion of subjects with titers of 1:40 or more on hemagglutination-inhibition assay at baseline was higher than expected. Among subjects who were 50 years of age or older, this finding could be attributed to the presence of preexisting antibodies from exposure to H1N1 viruses circulating before 1957.[23] It was surprising, however, to see higher baseline antibody titers in the younger age group. A number of factors could have contributed to the observed titers in both age groups at baseline. It is probable that there was some degree of previous 2009 H1N1 infection in the study population, despite stringent exclusion criteria. Cross-reactive antibodies to 2009 H1N1 may also have played a role. A study by Hancock et al. that analyzed stored-serum samples from trials of seasonal trivalent inactivated vaccine predating the current pandemic showed the presence

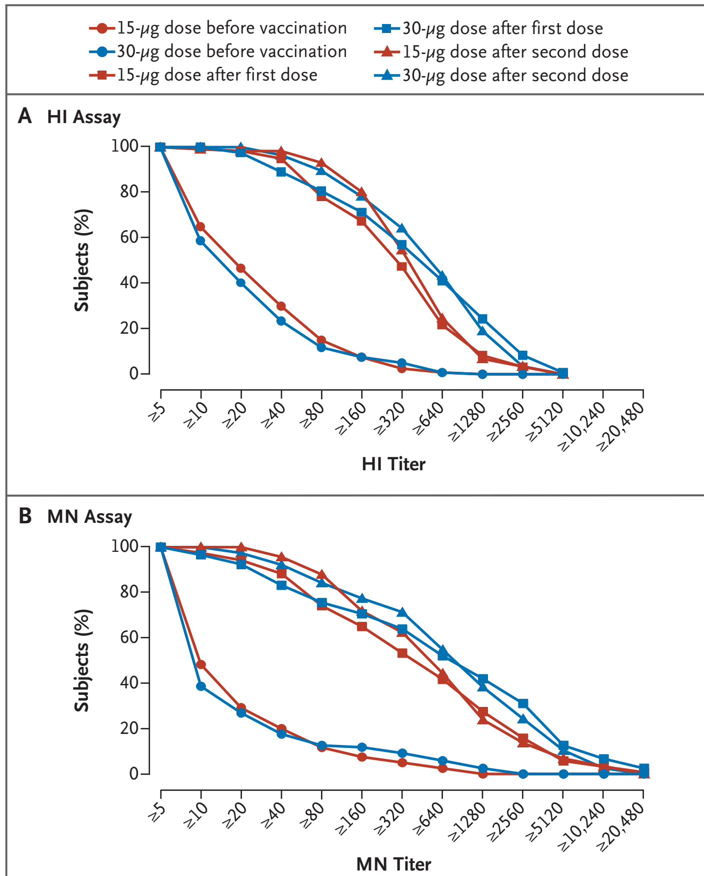

Figure 2. Reverse Cumulative Distribution Curves of Antibody Titers in Serum before Vaccination and 21 Days after the First and Second Dose of the H1N1 Vaccine, According to the Type of Assay.

Shown are levels of antibody titer against the 2009 H1N1 virus on hemagglutination-inhibition (HI) assay (Panel A) and on microneutralization (MN) assay (Panel B) before vaccination and after each of two doses of vaccine.

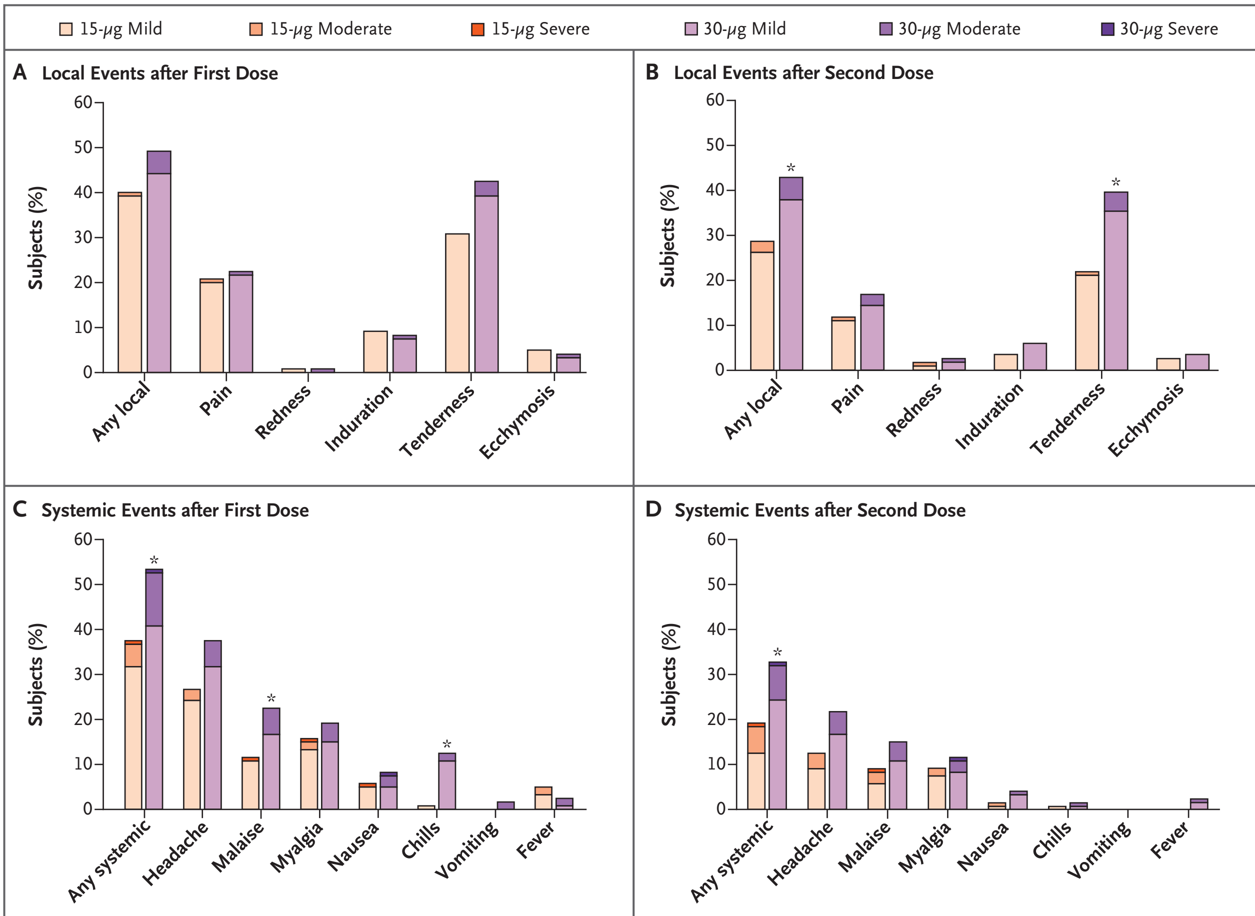

Figure 3. Solicited Reports of Adverse Events 7 Days after the First and Second Dose of the H1N1 Vaccine.
Shown are local adverse events associated with the H1N1 vaccine after the first dose (Panel A) and after the second dose (Panel B), as well as systemic adverse events (Panels C and D, respectively). The asterisks denote a significant ($P<0.05$) difference for the event between subjects receiving the 15-μg dose and those receiving the 30-μg dose.

of cross-reactive antibodies to 2009 H1N1 in adults.[24] The same study showed that vaccination with the seasonal vaccine resulted in a doubling in titers of these cross-reactive antibodies. The latter finding is particularly relevant, given that 45% of the subjects in our study had received the 2009 seasonal vaccine.

Even in subjects with no measurable antibodies at baseline, a single dose of vaccine elicited a robust immune response. The question remains: Why did these subjects have such a brisk response? The 2009 H1N1 pandemic differs from previous pandemics in that although the virus is antigenically very distant from recently circulating H1N1 viruses, it is still of the same H1N1 subtype.[25] Cross-protection that was afforded by exposure to antigenically drifted strains of the same influenza subtype has been described.[19] In addition, the 2009 H1N1 virus shares three gene sequences with the recently circulating seasonal H1N1 virus and three sequences with the current seasonal H3N2 virus.[23] Perhaps there is more immunotypic similarity between the 2009 H1N1 virus and recent seasonal strains than has been recognized previously.

The side-effect profile of the H1N1 vaccine, particularly the frequency and severity of solicited and unsolicited adverse events, is consistent with our previous experience with seasonal influenza vaccines in adults.[10] The full safety profile of H1N1 vaccine has not yet been elucidated. Population-based postlicensure surveillance will be required for all H1N1 vaccines, especially to assess rare outcomes, such as the Guillain–Barré syndrome.

Several important questions remain unanswered in this trial. First, since we studied healthy adults, trials need to be conducted in other populations that may have different responses to the vaccine, such as the elderly, children, and those with impaired immunity. Second, given the robust immune response to a 15-μg dose, lower antigen doses should be explored. Third, although our study is being carried out in one locality in Australia during winter in the Southern Hemisphere, our findings need to be borne out by studies in locations where the epidemiology of the pandemic may be different. Finally, estimates of the true effect of the vaccine when used in mass immunization programs will come from vaccine-effectiveness studies.

Supported by CSL with funding from the Department of Health and Aging of the Australian government.

All authors report being employees of CSL, and Dr. Greenberg, Dr. Lai, Dr. Hartel, Dr. Gittleson, Ms. Bennet, Ms. Dawson, Dr. Washington, and Dr. Basser report having an equity interest in the company. No other potential conflict of interest relevant to this article was reported.

We thank the subjects for their critical role in this study, the staff of CSL, and other staff participants, including the following: Dr. Sepehr Shakib and the staff at CMAX, a division of IDT Australia; the Clinical Trials Department at Focus Diagnostics in Cypress, CA; Quintiles of Australia; and Medidata of New York.

References

1. DG statement following the meeting of the Emergency Committee. Geneva: World Health Organization, 2009. (Accessed November 30, 2009, at http://www.who.int/csr/disease/swineflu/4th_meeting_ihr/en/index.html.)

2. Pandemic (H1N1) 2009 — update 63. Geneva: World Health Organization, 2009. (Accessed November 30, 2009, at http://www.who.int/csr/don/2009_08_28/en/index.html.)

3. Fiore AE, Shay DK, Broder K, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. MMWR Recomm Rep 2009;58(RR-8):1-52. [Erratum, MMWR Recomm Rep 2009;58:896-7.]

4. President's Council of Advisors on Science and Technology. Report to the president on U.S. preparations for 2009-H1N1 influenza. Washington, DC: White House, August 7, 2009. (Accessed November 30, 2009, at http://www.whitehouse.gov/assets/documents/PCAST_H1N1_Report.pdf.)

5. Kuehn BM. H1N1 vaccine. JAMA 2009;302:375.

6. Mathematical modelling of the pandemic H1N1 2009. Wkly Epidemiol Rec 2009;84:341-8.

7. Henderson DA, Courtney B, Inglesby TV, Toner E, Nuzzo JB. Public health and medical responses to the 1957-58 influenza pandemic. Biosecur Bioterror 2009;7:265-73.

8. World Health Organization. Availability of a candidate reassortant vaccine virus for the novel influenza A (H1N1) vaccine development. June 2009. (Accessed November 30, 2009, at http://www.who.int/csr/resources/publications/swineflu/ivr153_20090608_en.pdf.)

9. *Idem*. Summary of available candidate vaccine viruses for development of pandemic (H1N1) 2009 virus vaccines. July 2009. (Accessed November 30, 2009, at http://www.who.int/csr/resources/publications/swineflu/summary_candidate_vaccine.pdf.)

10. Talbot HK, Keitel W, Cate TR, et al. Immunogenicity, safety and consistency of new trivalent inactivated influenza vaccine. Vaccine 2008;26:4057-61.

11. Kendal AP, Pereira MS, Skehel JJ, eds. Concepts and procedures for laboratory-based influenza surveillance. Atlanta: Centers for Disease Control, 1982.

12. Rowe T, Abernathy RA, Hu-Primmer J, et al. Detection of antibody to avian influenza A (H5N1) virus in human serum by using a combination of serologic assays. J Clin Microbiol 1999;37:937-43.

13. CDC protocol of realtime RTPCR for influenza A (H1N1). (CDC reference no. I-007-05.) Geneva: World Health Organization, 2009. (Accessed November 30, 2009, at http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf.)

14. Committee for Proprietary Medicinal Products. Note for Guidance on Harmonisation of Requirements for Influenza Vaccines. London: European Medicines Agency, 1996. (Publication no. CPMP/BWP/214/96.) (Accessed November 30, 2009, at http://www.emea.europa.eu/pdfs/human/bwp/021496en.pdf.)

15. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for industry: clinical data needed to support the licensure of pandemic influenza vaccines. May 2007. (Accessed November 30, 2009, at http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091985.pdf.)

16. Neergaard L. Factory logjam could delay some swine flu shots. New York: Associated Press, August 18, 2009. (Accessed November 30, 2009, at http://news.aol.com/article/factory-logjam-could-delay-some-swine/448143.)

17. Englund JA, Walter EB, Gbadebo A, Monto AS, Zhu Y, Neuzil KM. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics 2006;118(3):e579-e585.

18. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. J Infect Dis 2006;194:1032-9.

19. Parkman PD, Hopps HE, Rastogi SC, Meyer HM Jr. Summary of clinical trials of influenza virus vaccines in adults. J Infect Dis 1977;136:Suppl:S722-S730.

20. Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. Emerg Infect Dis 2006;12:29-33.

21. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med 2006;354:1343-51.

22. Australian Government Department of Health and Ageing. Australian influenza surveillance report no. 11 — 8 to 24 July 2009. (Accessed November 30, 2009, at http://www.health.gov.au/internet/main/publishing.nsf/content/cda-ozflu-24-7-09.htm.)

23. Zimmer SM, Burke DS. Historical perspective — emergence of influenza A (H1N1) viruses. N Engl J Med 2009;361:279-85.

24. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med 2009;361:1945-52.

25. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 2009;325:197-201.