MAJOR ARTICLE

Immunogenicity of an Inactivated Monovalent 2009 H1N1 Influenza Vaccine in Pregnant Women

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Background. Although pregnant women are at increased risk of severe illness following influenza infection, there is relatively little information on the immunogenicity of influenza vaccines administered during pregnancy.

Methods. We conducted a clinical trial that enrolled 120 pregnant women in which participants were randomly assigned to receive an inactivated 2009 H1N1 influenza vaccine containing either 25 μg or 49 μg of hemagglutinin (HA) in a 2-dose series with a 21-day period between administration of the first and second doses.

Results. Following the first vaccination, HA inhibition (HAI) titers of ≥1:40 were detected in 93% (95% confidence interval [CI], 82%–98%) of subjects who received the 25-μg dose and 97% (95% CI, 88%–100%) of subjects receiving the 49-μg dose. In cord blood samples, HAI titers of ≥1:40 were found in 87% (95% CI, 73%–96%) of samples from the 25-μg dose group and in 89% (95% CI, 76%–96%) from the 49-μg dose group. Microneutralization titers tended to be higher than HAI titers, but the patterns of response were similar.

Conclusions. In pregnant women, 1 dose of an inactivated 2009 H1N1 influenza vaccine containing 25 μg of HA elicited an antibody response typically associated with protection against influenza infection. Efficient transplacental transfer of antibody was also documented.

Pregnant women are at increased risk of severe illness following infection with any influenza virus [1–3], but the risk is particularly great following infection due to the pandemic 2009 H1N1 influenza virus [4–19]. Reports from the 2009 pandemic indicate that pregnant women experienced an increased risk of hospitalization, were more likely to be admitted to the intensive care unit, and experienced increased mortality associated with 2009 H1N1 influenza infection, compared with nonpregnant women of similar age. Infection with the pandemic virus was also associated with neonatal complications, such as premature birth and adverse neonatal outcomes [11, 12, 20]. Pregnant women, who are routinely recommended to receive seasonal influenza vaccines, were targeted for priority receipt of monovalent 2009 influenza A (H1N1) vaccine during the 2009 H1N1 influenza pandemic [21, 22]. In addition, the possibility of continued or resurgent pandemic viral transmission led to the inclusion of the 2009 H1N1 influenza strain in the 2010–2011 trivalent seasonal influenza vaccine [23, 24].

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Several clinical trials have shown that a single dose of an inactivated, unadjuvanted 2009 H1N1 influenza vaccine is adequately immunogenic in nonpregnant adults [25–29] but, to our knowledge, there are no reported studies of these vaccines in pregnant women. Pregnancy is associated with immunologic changes, including the development of tolerance to foreign antigens and a decrease in total circulating immunoglobulin levels, which could decrease the immune response to vaccines [30–33]. Thus, immune responses to 2009 influenza A (H1N1) vaccines in nonpregnant adults may not predict responses in pregnant women.

To evaluate the immunogenicity of an inactivated pandemic H1N1 influenza vaccine in pregnant women, we conducted a prospective clinical trial in which pregnant women were randomized to receive 2 doses of 2009 H1N1 influenza vaccine containing either 25 μg or 49 μg of hemagglutinin (HA). To evaluate the immune responses to the vaccinations, blood samples were obtained prior to vaccination and at 21 days after each vaccination. To evaluate transplacental transfer of antibodies, maternal and cord blood samples were obtained at the time of delivery.

METHODS

Vaccine

The study vaccine was a monovalent, unadjuvanted, inactivated, subvirion, preservative-free preparation of the New York Medical College X-179A reassortant of the A/California/07/2009 H1N1 and PR8 strains recommended for use in pandemic vaccine development by the World Health Organization [34]. Seed virus was propagated in embryonated chicken eggs, inactivated, and split in accordance with the process used by the manufacturer (Sanofi Pasteur) to produce licensed seasonal influenza vaccine.

The study was designed to evaluate vaccine doses of 15 μg and 30 μg of HA. Prior to release of the clinical lots for the trial, potency testing performed using a high-performance liquid chromatography (HPLC) assay estimated an HA concentration of 30 μg per mL; based on that information, injected volumes of 0.5 mL or 1.0 mL were administered to study participants randomized to the 15-μg or 30-μg dose groups, respectively. The HPLC assay was used instead of the standard single radial immunodiffusion (SRID) potency assay, because calibration reagents needed for the SRID assay were unavailable at the time the clinical lots were formulated. Subsequent retesting of the clinical lots with the standard SRID assay showed the actual HA content of the study vaccine to be 25 μg and 49 μg for volumes of 0.5 mL and 1.0 mL, respectively.

Study Design

Pregnant women 18–39 years of age who were in their second or third trimester (14–34 weeks gestation) were screened for eligibility and provided written informed consent (for eligibility criteria, see the Supplementary Appendix). At enrollment, participants were asked about prior receipt of the 2008–2009 and the 2009–2010 seasonal influenza vaccines. Women who had received the inactivated 2009–2010 seasonal influenza vaccine were eligible for enrollment if at least 2 weeks had elapsed since administration of that vaccine.

Eligible subjects were randomly assigned with equal probability to receive either the lower dose (0.5 mL) or the higher dose (1.0 mL) of study vaccine. The second dose of the same vaccine was given 21 days after the first. Vaccinations were given intramuscularly in the deltoid by a member of the study team who was not involved in the subsequent assessment of adverse events, and the contents of the syringe were shielded, to the extent possible, from the subject's view.

Following each vaccination, subjects were provided with a memory aid to record the presence and severity of local signs and symptoms (pain, tenderness, redness, and swelling), systemic symptoms (feverishness, malaise, myalgia, headache, and nausea), and oral temperature on the evening of vaccination and for the next 7 days. Participants were instructed to grade reported symptoms as mild if they did not interfere with daily activities, moderate if they resulted in some interference with daily activities, and severe if they prevented subjects from engaging in daily activities. Pain that did not interfere with normal activities but required the use of pain medications was defined as moderate. Unsolicited adverse events were collected through 21 days after the second vaccination and serious adverse events (SAEs), which included pregnancy outcomes, complications of labor and delivery, and neonatal outcomes, were collected through the last follow-up visit at 201 days after enrollment.

Blood samples were obtained before the first vaccination (baseline), before the second vaccination (21 days after the first vaccination), and at 21 days after the second vaccination. Another blood sample was obtained from the participant during the delivery hospitalization but prior to delivery, and a cord blood sample was obtained at the time of delivery.

Laboratory Assays

Microneutralization (MN) and hemagglutination inhibition (HAI) assays were performed at the Southern Research Institute laboratory using the A/California/07/2009 (H1N1) influenza virus and according to established procedures [35, 36]. The HAI assay is the most commonly used assay to assess the immune response to influenza vaccines and was used in part to have the ability to compare results with trials of influenza vaccines in healthy young adults. The microneutralization assay was used to assess the function of the antibody generated in response to vaccination. The HAI assays were performed using turkey erythrocytes with removal of nonspecific inhibitors of agglutination using receptor-destroying enzyme. The serum samples were tested at an initial dilution of 1:10, and laboratory personnel were blinded to sample identity.

Statistical Analysis

The 2 coprimary immunologic end points were defined at day 21 following the first vaccination and included the proportion of subjects who had an HAI titer of ≥1:40 and the proportion of subjects who met the definition of seroconversion by HAI (either a ≥4-fold increase in titer from baseline or a postvaccination titer ≥1:40 if the baseline titer was <1:10). The endpoints were also evaluated for titers determined by the MN assay. For analyses of the results of both assays, titers below the limit of detection were assigned a value of 5. Exact (Clopper-Pearson) confidence intervals (CIs) are reported for the endpoints of postvaccination titer ≥1:40 and the proportion of subjects who met the definition of seroconversion. Comparisons of proportions between groups were performed using Fisher's exact test.

Geometric mean titers (GMTs) were calculated by transforming data to log scale for all computations and comparisons and transforming these results back to the original scale. Comparisons between groups were performed with the use of the *t* test. Unadjusted *P* values are reported if <.05, which indicated statistical significance. *P* values >.05 are not reported.

The sample size of 60 subjects per study group was selected to provide information on the dose-related immune response in a timely fashion. This sample size was not based on formal power calculations because the study was not designed to test a specific null hypothesis. However, the study was designed to generate descriptive data supportive of the hypothesis that the H1N1 inactivated influenza vaccine would be well tolerated and would elicit adequate immune responses among pregnant women. The sample size was also based on logistical considerations, such as the ability to complete recruitment in a timely fashion and a review of the precision of resulting estimates based on a range of likely outcomes. Power computations performed a priori determined that the sample size of 60 subjects per group yielded 80% power (with alpha of 0.05) to detect a difference in proportions, such as the seroconversion rate or proportion of subjects with a titer of ≥1:40, in the range of 15%–25%.

The study was approved by the institutional review boards of record of each of the participating study sites. The vaccine manufacturer provided the study product but had no role in the conduct of the study, analysis of the data, or preparation of this report.

RESULTS

Participants were enrolled from 9 September 2009 through 16 October 2009. During this period, each of the 5 states in which subjects were enrolled reported ≥3 weeks of widespread influenza activity [37]. A total of 121 subjects were enrolled; of these, 120 received the first vaccination, and 103 received the second vaccination. The characteristics of the 120 subjects given a first vaccination are shown in Table 1. The subjects' ages, demographic characteristics, mean gestational age at enrollment, and the proportion of subjects in the second or third trimester were not significantly different between the 2 dose groups.

Safety Analyses

Both vaccine dose levels were generally well tolerated (Table 2). Local injection site symptoms of pain and tenderness were more common in the 49-μg dose group following both the first and second vaccinations; however, the differences between the dose groups were not statistically significant, and nearly all of the reactions were mild in severity. Within each dose group, there was no significant change in the frequency of reported local reactions between the first and second vaccinations. The frequency of occurrence of systemic symptoms did not vary between the 2 dose groups or between the first and second vaccinations within each dose group.

Eighteen SAEs were reported for 15 women, and 24 SAEs were reported for 20 infants; all were considered to be unrelated to the vaccine, and the frequency of events was generally balanced across study groups, with 9 of the 15 maternal SAEs and 13 of the 20 infant SAEs reported in the 25-μg dose group. The 15 maternal SAEs included 6 reports of postpartum hemorrhage, 2 reports of preterm contractions, 2 reports of severe preeclampsia, and 1 report each for the outcomes of abdominal myomectomy, exacerbation of asthma, gestational hypertension at term, fetal loss at 20 weeks gestation, nonelective Cesarean section, premature delivery, retained placenta, and vaginal bleeding. The 24 infant SAEs included 5 reports of premature birth, 4 reports of sacral dimple, 3 reports of atrial septal defect, and 1 report each of congenital heart disease, Erb's palsy, fetal demise at 36 weeks gestational age, hyperbilirubinemia, possible Hirschsprung's disease, postaxial polydactyly, pulmonic stenosis, respiratory distress, simple complete syndactyly, tetralogy of Fallot, thickened nuchal fold, and fetal distress resulting in an emergency Cesarean section.

Immunogenicity Analyses

At baseline, most participants were seronegative for the 2009 H1N1 influenza virus (Table 3). Following the first vaccination, an HAI antibody titer of ≥1:40 was detected in 93% (95% CI, 82%–98%) of subjects who received the 25-μg vaccine and 97% (95% CI, 88%–100%) of subjects who received the 49-μg vaccine, with GMTs of 384.2 (95% CI, 259.6–568.6) and 460.7 (95% CI, 325.2–652.7) in the 25-μg and 49-μg dose groups, respectively. These differences were not statistically significant. Microneutralization antibody titers were higher than HAI titers, with GMTs of 444.1 (95% CI, 309.7–636.7) and 595.7 (95% CI, 443.5–800.2) in the 25-μg and 49-μg dose groups, respectively, but as with the HAI titers, there were no significant differences between the dose groups for any of immunogenicity endpoints (proportion with titer ≥1:40, proportion meeting the definition

Table 1. Characteristics of Study Subjects at Enrollment

Characteristic	Dose group	
	25 μg ($n = 60$)	49 μg ($n = 60$)
Age, mean years (range)	31.7 (20, 39)	31.2 (18, 39)
Trimester of gestation, %		
Second (14–26 weeks)	57	72
Third (27–34 weeks)	43	28
Gestational age at enrollment, mean weeks (± standard deviation)	24.4 ± 6.2	22.6 ± 6.0
Race, %		
White	85	80
Black	3	7
Asian	7	10
Other	5	3
Non-Hispanic ethnicity, %	88	93
Received 2008–2009 influenza vaccine,[1] %	68	58
Received 2009–2010 influenza vaccine,[2] %	28	27

NOTE. [1] The 2008–2009 influenza vaccine contained A/Brisbane/10/2007 (H3N2)-like, A/Brisbane/59/2007 (H1N1)-like, and B/Florida/4/2006-like strains.
[2] The 2009–2010 influenza vaccine contained A/Brisbane/10/2007 (H3N2)-like, A/Brisbane/59/2007 (H1N1)-like, and B/Brisbane/60/2008-like strains.

of seroconversion, or postvaccination GMT) following the first vaccination. There were no significant increases in GMT, either by HAI or MN assays, following the second vaccination.

At delivery, 85% (95% CI, 71%–94%) of women who had received the 25-μg vaccine had an HAI antibody titer of ≥1:40, but this level was detected in only 62% (95% CI, 46%–75%) of women who had received the 49-μg dose ($P = .02$). The difference in proportion with titer ≥1:40 at delivery was less pronounced for MN titers. Similarly, the GMTs in the maternal delivery samples were significantly higher in the 25-μg dose group compared with the 49-μg dose group by HAI (132.1 vs 50.9; $P = .01$), but not by MN (333.8 vs 191.0; $P = .14$).

Cord blood HAI and MN GMTs were also higher in the 25-μg dose group compared with the 49-μg dose group, but these differences were not statistically significant. Cord blood HAI GMTs were higher than maternal delivery sample GMTs in both dose groups, and this difference was statistically significant for the 49-μg dose group ($P = .002$). The geometric mean ratio (GMR) of cord blood to maternal blood HAI titers was 1.81 (95% CI, 1.48–2.21) in the 25-μg group and 2.96 (95% CI, 2.16–4.06) in the 49-μg group. The GMR for MN titers was 1.52 (95% CI, 1.24–1.86) in 25-μg group and 1.60 (95% CI, 1.30–1.96) in the 49-μg group. In analyses of paired maternal delivery and cord blood samples, the cord blood titer was higher than the corresponding maternal delivery sample titer in most pairs (Figure 1).

The panels in Figure 2 display the relationships between the variables of maternal age at first vaccination and interval in days between the mother's second vaccination and delivery with the endpoints of HAI and MN antibody titers in cord blood and maternal delivery samples. There was no relationship between mother's age and these endpoints. The downward slopes in the plots of the relationship of the interval from the second vaccination and delivery suggest trends toward lower cord blood and maternal delivery titers with vaccination earlier in pregnancy. This relationship was confirmed in analyses of covariance that adjusted for dose group. Those analyses found significant associations between the interval in days variable and the endpoints of HAI and MN log titer in cord blood and maternal delivery samples ($P < .05$ for all 4 analyses).

In analyses stratified by prior receipt of the 2008–2009 and/or 2009–2010 seasonal influenza vaccines, there was no evidence of a lower response to the first or second vaccination among participants reporting prior receipt of seasonal influenza vaccine (Table 4).

There was good correlation between the results of the HAI and MN assays in postvaccination samples. The Spearman correlation coefficient for the HAI and MN titers among all participants prior to vaccination was 0.34 and increased to 0.81 for the post–dose 1 and post–dose 2 visits. The correlation between the 2 assays for the vaccine group-specific dose responses was similar.

DISCUSSION

This evaluation of the immunogenicity of a 2009 H1N1 influenza vaccine in pregnant women indicates that a single dose of an inactivated 2009 H1N1 influenza vaccine is highly immunogenic in women vaccinated during the second or third trimester of pregnancy. At 3 weeks after administration of a 25-μg dose, 93% of women had an HAI titer of ≥1:40, which is a level typically associated with protection against influenza

Table 2. Solicited Local and Systemic Adverse Effects During the Week After Vaccination

Effect	Vaccinated subjects, %			
	First vaccination, 25 μg (n = 60)	First vaccination, 49 μg (n = 60)	Second vaccination, 25 μg (n = 49)	Second vaccination, 49 μg (n = 54)
Local reactions[1]				
Pain at injection site				
None	75	65	80	63
Mild	23	35	20	35
Moderate	2	0	0	2
Tenderness				
None	57	38	47	31
Mild	40	60	53	69
Moderate	3	2	0	0
Erythema				
None	92	87	96	94
<20 mm	8	10	4	4
≥20 and <50 mm	0	3	0	2
Swelling/induration				
None	93	98	98	100
<20 mm	5	2	0	0
≥20 and <50 mm	2	0	2	0
Systemic reactions				
Fever[2]				
None	92	93	96	93
Mild	7	5	2	7
Moderate	2	2	2	0
Malaise[2]				
None	68	60	84	74
Mild	23	27	4	15
Moderate	8	13	12	11
Myalgia				
None	80	87	94	89
Mild	12	10	4	7
Moderate	7	3	2	4
Severe	2	0	0	0
Nausea[2]				
None	83	80	96	93
Mild	12	15	0	7
Moderate	5	5	4	0
Headache				
None	72	70	78	80
Mild	23	27	16	20
Moderate	5	3	4	0
Severe	0	0	2	0
Oral temperature[2]				
<37.8°C	100	98	98	100
≥37.8°C and <38°C	0	2	0	0
≥38°C and <39°C	0	0	2	0

NOTE. [1] No severe local reactions were reported.

[2] No severe reactions were reported.

infection. There was no further increase in HAI GMTs with the second vaccination, which suggested no apparent benefit of a second vaccination for the mother. The vaccinations were well tolerated, with reactogenicity profiles that were similar to those reported for 2009 H1N1 influenza vaccines in nonpregnant adults [25, 26, 28, 38].

Table 3. Serum Hemagglutination Inhibition and Microneutralization Assay Responses Before and After Each Dose of the 2009 H1N1 Influenza Vaccine

	Hemagglutination inhibition assay		Microneutralization assay	
Immunogenicity end point	25-μg vaccine dose	49-μg vaccine dose	25-μg vaccine dose	49-μg vaccine dose
Titer ≥1:40, % (95% CI)				
Baseline	7 (2–18)	7 (2–17)	13 (5–24)	16 (7–27)
21 Days after dose 1	93 (82–98)	97 (88–100)	96 (87–100)	97 (88–100)
21 Days after dose 2	95 (82–99)	92 (81–98)	100 (91–100)	100 (93–100)
Delivery				
Maternal	85 (71–94)	62 (46–75)[1]	95 (83–99)	87 (74–95)
Cord blood	87 (73–96)	89 (76–96)	92 (79–98)	91 (79–98)
Seroconversion, % (95% CI)				
21 Days after dose 1	89 (78–96)	97 (88–100)	93 (82–98)	97 (88–100)
21 Days after dose 2	95 (82–99)	92 (81–98)	97 (86–100)	100 (93–100)
Geometric mean titer (95% CI)				
Baseline	6.8 (5.3–8.7)	6.3 (5.1–7.8)	9.5 (7.3–12.4)	9.6 (7.5–12.3)
21 Days after dose 1	384.2 (259.6–568.6)	460.7 (325.2–652.7)	444.1 (309.7–636.7)	595.7 (443.5–800.2)
21 Days after dose 2	360.3 (225.0–577.0)	347.2 (233.3–516.6)	509.5 (349.6–742.6)	543.7 (413.1–715.5)
Delivery				
Cord blood	230.3 (152.1–348.7)	150.3 (100.9–223.9)[2]	503.5 (329.6–769.1)	299.3 (197.7–453.2)
Maternal	132.1 (83.0–210.3)	50.9 (30.5–84.9)[1]	333.8 (216.5–514.6)	191.0 (121.3–300.6)
Geometric mean ratio of cord blood:maternal delivery titer (range)	1.81 (1.48–2.21)	2.96 (2.16–4.06)	1.52 (1.24–1.86)	1.60 (1.30–1.98)

NOTE. The number of specimens tested by the hemagglutination inhibition assay for the 25-μg dose group included 55 specimens obtained at baseline and after dose 1, 38 specimens obtained after dose 2, 41 maternal delivery specimens, and 39 cord blood specimens. For the 49-μg dose group, the number of specimens included 58 specimens obtained at baseline and after dose 1, 51 specimens obtained after dose 2, 47 maternal delivery specimens, and 46 cord blood specimens. The number of specimens tested by the microneutralization assay for the 25-μg dose group included 55 specimens obtained at baseline and after dose one, 38 specimens obtained after dose 2, 41 maternal delivery specimens, and 39 cord blood specimens. For the 49-μg dose group, the number of specimens included 58 specimens obtained at baseline and after dose 1, 51 specimens obtained after dose 2, 47 maternal delivery specimens, and 45 cord blood specimens. CI, confidence interval.

[1] $P \leq .02$ for comparison of 25-μg dose group with 49-μg dose group.

[2] $P = .002$ for comparison of cord blood geometric mean titer and maternal delivery geometric mean titer.

We followed women through the time of delivery, to evaluate persistence of the immune response in the vaccinated women and the transplacental transfer of antibody, as determined by antibody levels in infant cord blood samples, compared with samples obtained from the mother at the time of delivery. We found that, although antibody titers in the vaccinated women decreased in the interval between vaccination and delivery, relatively high titers were maintained up to the time of delivery, and cord blood titers tended to be higher than maternal delivery titers. These findings suggest that clinical protection from vaccination during pregnancy may persist to the postpartum period and can be efficiently transferred to the infants.

The relatively high cord blood titers are consistent with clinical benefits reported in 3 recent evaluations of inactivated seasonal influenza vaccines given during pregnancy. A clinical trial of influenza vaccine given to women in Bangladesh during their third trimester of pregnancy reported that an HAI titer of ≥1:40 to the vaccine influenza A H1N1 strain was detected in >80% of maternal delivery and cord blood samples [39] and that influenza vaccination during pregnancy was associated with a reduction in the risk of febrile illness in both the women and their infants [40]. An observational cohort study of 1160 mother-infant pairs in the United States found that infants born to mothers who had received influenza vaccine during pregnancy had a 41% lower risk of laboratory-confirmed influenza infection than did infants born to mothers who had not been vaccinated [41]. Similarly, a case control study found that infants whose mothers were vaccinated during pregnancy were 91% less likely to be hospitalized with influenza infection during the first 6 months of life than were infants whose mothers had not been vaccinated [42].

We found consistent trends toward higher HAI antibody levels in cord blood samples, compared with maternal delivery samples. Other evaluations of transplacental transfer of maternal antibodies have found higher levels of antibodies to protein antigens, such as pertussis antigens, and to pneumococcal antigens, such as pneumococcal polysaccharide, in cord blood samples than in maternal delivery specimens, which is believed to be the result of

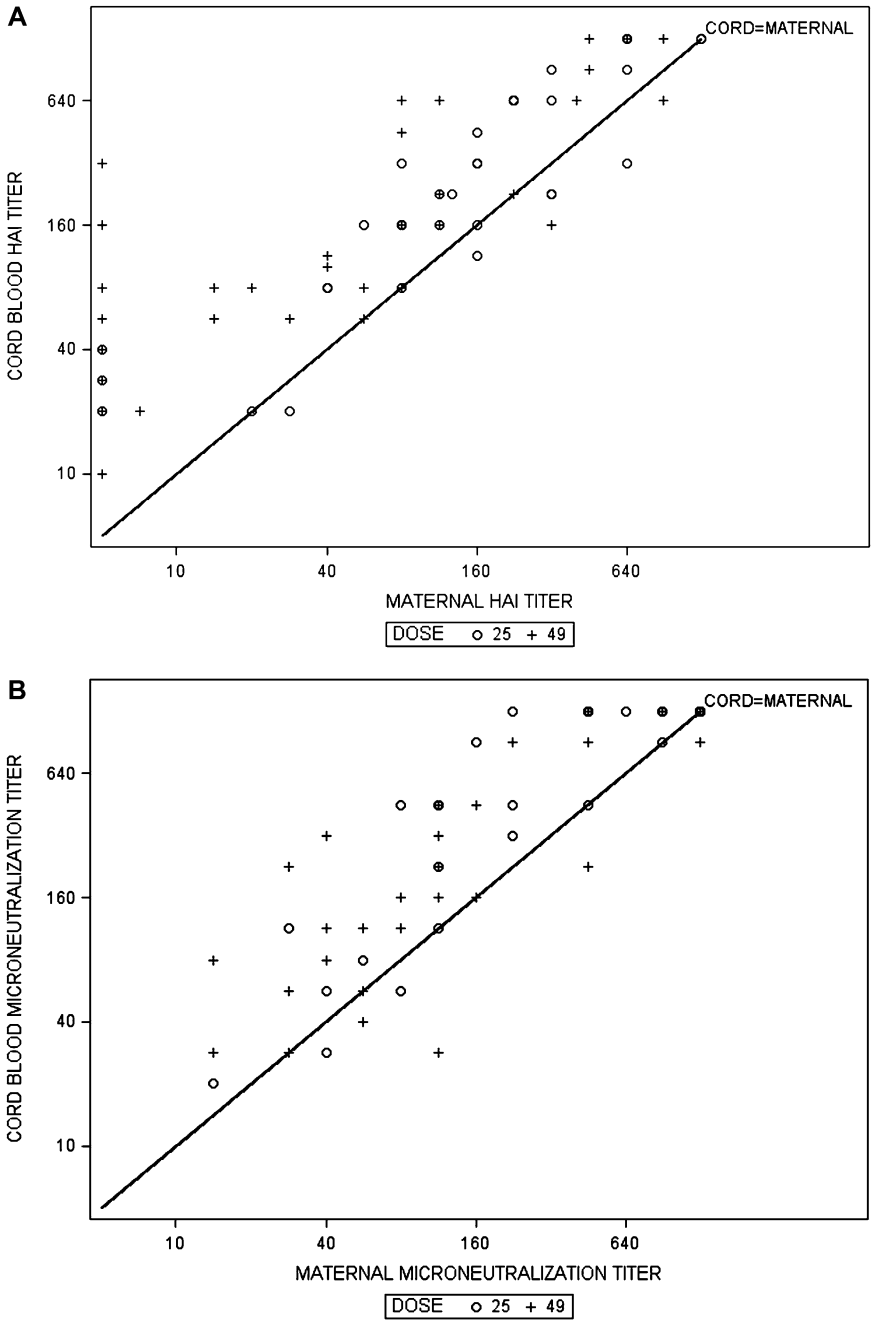

Figure 1. Comparison of antibody titers by hemagglutination inhibition (HAI) (*A*) and microneutralization (*B*) assays in maternal delivery and infant cord blood pairs, by dose group (*circles*, 25 μg; *crosses*, 49 μg). The diagonal lines indicate equal values for the paired maternal delivery and infant cord blood specimens. Points above the line indicate a cord blood titer higher than the maternal delivery titer within each pair.

active placental transport of maternal antibody [43–46]. Interestingly, this phenomenon has not been previously demonstrated in evaluations of maternal influenza vaccination. In fact, a recent evaluation of an MF59-adjuvanted 2009 H1N1 influenza vaccine given in the third trimester of pregnancy found that HAI GMTs were significantly lower in the cord blood samples than in the maternal delivery samples (141.8 vs 257.9) [47]. Similarly, in the clinical trial of seasonal influenza vaccine conducted in Bangladesh, HAI GMTs in the cord blood specimens were either lower than or comparable to those detected in the maternal delivery samples [39]. Finally, in the observational study of seasonal influenza vaccines reported by Eick et al [41], HAI GMTs to vaccine strains in the cord blood samples were generally similar to those in maternal postpartum specimens. Our findings could represent chance observations, they may be a function of the administration of 2 doses of a higher–antigen content vaccine, or they may be attributable to other factors. We also found that vaccination later in pregnancy may be associated with higher cord

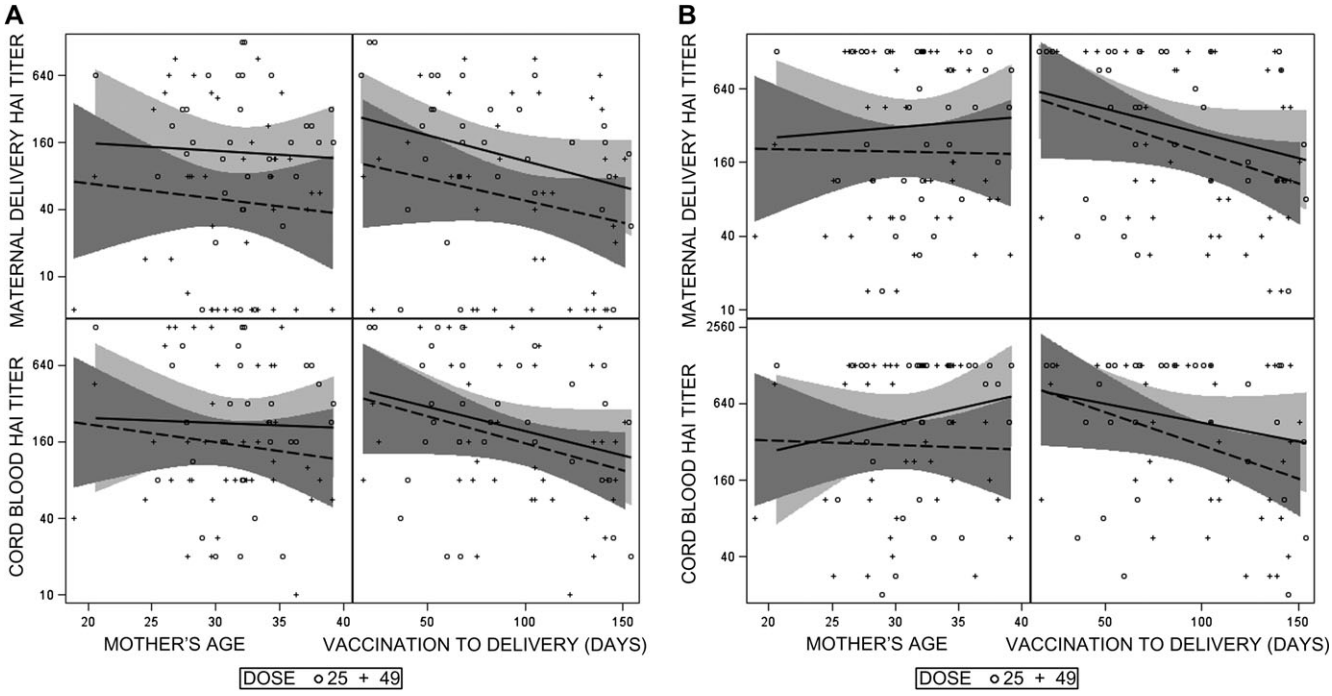

Figure 2. Relationships between the variables of mother's age in years at the first vaccination and interval in days between the second vaccination and delivery with the endpoints of cord blood and maternal delivery titers by hemagglutination inhibition (HAI) (*A*) and microneutralization (MN) (*B*) assays. The markers indicate the individual values for the 25-μg (*circles*) and 49-μg (*crosses*) dose groups, the lines are the estimated linear regression lines for each dose group (*solid line*, 25 μg; *dashed line*, 49 μg), and the shaded areas indicate the 95% confidence bands for each regression line.

blood antibody levels, which could have implications for the level of clinical protection or duration of persistence of passively acquired antibody in the neonates.

There are limitations to this study that may be relevant to the interpretation of the results. First, we enrolled only women in the second and third trimesters of pregnancy and so did not evaluate antibody response to vaccines administered earlier in pregnancy. Second, we did not collect information on height and weight of the pregnant women and so could not evaluate the possible influence of body mass index on vaccine response. Last, as noted in the methods, because of issues with the potency testing of the vaccine, we evaluated doses of 25 μg and 49 μg of HA, instead of the intended doses of 15 μg and 25 μg of HA.

Although we were not able to evaluate a dose of 15 μg of HA (the standard dose per strain contained in licensed inactivated influenza vaccines), our results suggest that inactivated vaccines containing 15 μg of 2009 H1N1 influenza hemagglutinin are likely to be adequately immunogenic in pregnant women. In dose-ranging studies of seasonal influenza vaccines and 2009 H1N1 influenza vaccines involving nonpregnant adults, the proportion of subjects who achieved an HAI titer of ≥1:40 appears to be relatively insensitive to dose [25–29, 48–50]. For

Table 4. Geometric Mean Hemagglutination Inhibition and Microneutralization Titers to the 2009 H1N1 Influenza Vaccine According to Prior Receipt of 2008–2009 and/or 2009–2010 Seasonal Influenza Vaccines

Assay	Dose group	Received 2008–2009 and/or 2009–2010 seasonal influenza vaccine	No. of subjects	Day 0, GMT (95% CI)	No. of subjects	Day 21 after dose 1, GMT (95% CI)	No. of subjects	Day 21 after dose 2, GMT (95% CI)
HAI	25 μg	No	10	5.2 (4.8–5.6)	10	355.1 (142.5–884.9)	8	349.0 (123.8–983.4)
		Yes	44	7.2 (5.3–9.8)	44	380.5 (240.8–601.4)	29	347.9 (196.1–617.2)
	49 μg	No	17	5.8 (4.3–7.8)	17	417.1 (225.3–772.1)	14	430.7 (264.0–702.5)
		Yes	40	6.7 (5.0–8.9)	40	489.3 (312.8–765.2)	36	320.0 (186.2–549.9)
MN	25 μg	No	10	5.0 (…)[1]	10	320.0 (129.4–791.2)	8	293.4 (106.6–807.8)
		Yes	44	11.0 (8.0–15.0)	44	467.0 (309.1–705.6)	29	574.7 (376.5–877.3)
	49 μg	No	17	6.8 (4.5–10.3)	17	461.9 (257.1–829.7)	14	551.7 (320.8–948.7)
		Yes	40	11.3 (8.3–15.4)	40	662.6 (462.2–949.8)	36	543.4 (385.3–766.4)

NOTE. CI, confidence interval.

[1] All samples had the same value (below the limit of detection of a titer of 1:10), and therefore confidence intervals could not be calculated.

example, in a study of an inactivated 2009 H1N1 vaccine made by Sanofi Pasteur in a cohort of adults 18–64 years of age, a postvaccination titer of ≥1:40 was detected in 95% of subjects given a vaccine containing 11 μg of antigen, compared with 98% of subjects given a vaccine containing 24 μg of antigen [28]. Thus, based on these findings, it is highly likely that a single dose of vaccine containing 15 μg of HA given to pregnant women would induce antibody titers typically correlated with protection and that transplacental transfer of antibody would confer passive protection to the newborns.

Supplementary Data

Supplementary data are available at *The Journal of Infectious Diseases* online.

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