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Immunogenicity of Trivalent Influenza Vaccine in Extremely-Low-Birth-Weight, Premature versus Term Infants

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Abstract

Background—Influenza vaccine immunogenicity in premature infants is incompletely characterized.

Objective—To assess the immunogenicity of trivalent, inactivated influenza vaccine (TIV) in extremely low-birth-weight (ELBW, ≤ 1000 grams birth weight), premature (< 30 weeks gestation) infants. We hypothesized that geometric mean titers (GMT) of influenza antibody would be lower in premature than in full-term (≥ 37 week) infants.

Design/Methods—In this prospective, multicenter study, former premature and full-term infants ages, 6–17 months, received 2 doses of TIV during the 2006–7 or 2007–8 influenza seasons. Sera were drawn before dose 1 and 4–6 weeks after dose 2. Antibody was measured by hemagglutination inhibition.

Results—Over two years, 41 premature and 42 full-term infants were enrolled; 36 and 33 of these infants, respectively, had post-vaccination titers available. Premature infants weighed less (mean 1.3 – 1.8 kg difference) at the time of immunization than full-term infants. Pre-vaccination titers did not differ between groups. Premature infants had higher post-vaccination antibody GMT than full-term infants to H1 (2006–7, 1:513 v. 1:91, $P=0.03$; 2007–8, 1:363 v. 1:189, $P=0.02$) and B/Victoria (2006–7, 1:51 v. 1:10, $P=0.02$). More premature than full-term infants had antibody titers $\geq 1:32$ to B/Victoria (85% v. 60%, $p=0.04$) in 2007–8. Two (5%) premature and 8 (19%) full-term infants had adverse events, primarily fever, within 72 hours after vaccination. No child had medically-diagnosed influenza.

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Conclusions—Former premature infants had antibody responses to two TIV doses greater than or equal to those of full-term children. Two TIV doses are immunogenic and well tolerated in ELBW, premature infants 6–17 months old.

Keywords

Premature infant; very low birth weight infant; influenza vaccines; immunization; vaccines

INTRODUCTION

Influenza is a leading cause of death in children worldwide.¹ Severe influenza respiratory disease is increasingly recognized in children in the United States.^{2, 3} During the 2003–2004 influenza season, 153 influenza-associated deaths were reported among U.S. children.⁴ Premature infants are over-represented among children < 5 years old hospitalized for respiratory illnesses.⁵ Influenza hospitalization rates in high-risk children under 2–5 years of age, including premature infants and those with chronic lung diseases such as bronchopulmonary dysplasia (BPD), are increased 3-to-5-fold over rates among other children.^{5–8} Premature births currently account for 12.7% of all births in the US, or over 500,000 births per year,⁹ and thus represent a substantial population who could be at increased risk for more severe influenza infection before age 5 years.

Although vaccines against influenza have been available for decades, their efficacy in children ≤ 5 years old may be less than 50%.^{10, 11} Several studies have reported that the effectiveness of the trivalent inactivated influenza vaccine (TIV) is particularly low in children <2 years old, for whom it is the only licensed vaccine.^{12–15} Influenza vaccines have been incompletely studied in premature infants. Studies performed approximately 15 years ago suggested lower antibody responses in former premature infants ≤ 18 months old than in full-term children of the same age,^{16, 17} but a more recent study suggested that premature infants born at 22–35 weeks' gestation, aged 6–11 months, had antibody responses similar to those of historical, full-term controls.¹⁸

We prospectively assessed antibody responses to TIV among former extremely-low-birth-weight (ELBW), premature infants compared with full-term infants during two successive influenza seasons. The study focused specifically on ELBW infants and used concurrent, rather than historical, controls. We hypothesized that the geometric mean titer (GMT) of antibody to each of the three vaccine components would be lower among premature infants than among full-term infants following receipt of their first series of TIV.

MATERIALS AND METHODS

We enrolled infants in a prospective-cohort immunogenicity study during the 2006–7 and 2007–8 influenza vaccine seasons at five sites comprising the Premature Infant Vaccine Collaborative. The study was approved by the institutional review board of each site. Each child was included in the study during only one season. To be included in the study, children were required to be 6–17 months chronological age and not to have received prior influenza immunization. The age range was chosen to include children old enough to be eligible to receive vaccine (i.e. ≥ 6 months old), but still in the first influenza season during which they were eligible for vaccine. Children were included in the premature group if they were <30 weeks' gestation and weighed ≤ 1000 grams at birth, or in the full-term group if they were 37–42 weeks' gestation and weighed >2500 grams at birth. Informed, written parental permission, agreement of the primary care provider and the ability to return for study visits were required for all subjects. Children with known contraindication to influenza

immunization, known immunodeficiency, systemic glucocorticoid therapy at the time of study enrollment, or medically-diagnosed influenza prior to enrollment were excluded.

Children had three study visits. Children received two 0.25 mL doses of intramuscular, trivalent, inactivated, split-virion influenza vaccine (Fluzone®, Sanofi Pasteur, Swiftwater, PA), 4–6 weeks apart (Visits 1 and 2). Vaccine was purchased commercially at each site. The components of the vaccine were A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004 in the 2006–7 season, and A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2) and B/Malaysia/2506/2004 in the 2007–8 season. The vaccines contained 7.5 mcg of each of the 3 hemagglutinins per 0.25 mL dose. Children had blood samples (1 mL) drawn at the time of the first dose of vaccine and 4–6 weeks following the second dose of vaccine (Visit 3). Their medical history was reviewed at each study visit. Vaccine reaction data were collected for the 72 hours following each vaccine dose, using a parent diary, which was reviewed and confirmed by a phone call from study staff at 72–96 hours following each vaccine dose. Other adverse events occurring since the previous visit were determined at each study visit. Adverse event information was collected through Visit 3. Birth and past medical history and suspected severe adverse events (life-threatening or resulting in hospitalization, disability or death) were confirmed by primary record review. Events consistent with known vaccine reactions (e.g. local reactions, fever, mental status changes) and occurring within 72 hours after vaccine administration were considered likely to be vaccine-related.

Sera were separated by centrifugation within 4 hours, and stored at –80°C at each site until the close of enrollment. Serum samples were shipped to the University of Rochester on dry ice by overnight courier for analysis. Influenza antibody titers were measured by microtiter hemagglutination inhibition (HAI) test.¹⁹ Reagents for the HAI test were obtained from the World Health Organization Collaborating Centers for Influenza, US Centers for Disease Control and Prevention. Briefly, the hemagglutination titer for each influenza antigen was determined and diluted to contain 4 hemagglutinin units in 0.025 mL of the virus suspension. H1, H3, B/Victoria and B/Yamagata (not represented in the vaccine either year) antigens were used. Subject serum samples were heat inactivated and treated with Receptor-destroying Enzyme (RDE, Accurate Chemical Supply) to remove non-specific inhibitors. For each viral antigen to be tested, serial 2-fold dilutions of children's sera were prepared, and 4 HA units of antigen and 0.5% guinea pig red blood cell suspension were added to each dilution. Serum titers were determined by the highest dilution of serum capable of inhibiting hemagglutination. Titers were confirmed by back-titration and a serum control was run on each plate. All specimens were analyzed in duplicate and all assays were run using the same lot of antigen. Laboratory personnel were blinded as to the timing and identity of the samples.

Study data were first recorded on paper case report forms and then transcribed into a web-based data management system by a study coordinator at each site. Quality assurance checks on data type, value range, and completeness were performed through the data management system as well as by regular manual reviews by the data manager and statistician. Serology data were integrated before analyses were performed.

The primary outcome was the GMT of antibody, as measured by HAI, to each component of the influenza vaccine. Since the 2006–7 enrollment was intended primarily to provide estimates of the titers of antibody achieved by premature and full-term infants following influenza vaccination, no formal sample size estimation was undertaken. The 2007–8 enrollment was designed to test the differences in influenza GMT between premature and full-term groups. The 2007–8 enrollment goal (46 subjects per group) was designed to provide 80% power at 5% significance level to detect a 1.5-fold difference in GMT between

groups, assuming a standard deviation (SD) spanning 0.5 to 2.0 times the value of each GMT, using a two-sided t test. The secondary outcome was the proportion of children achieving an HAI antibody titer $\geq 1:32$ to each component of the influenza vaccine, a value often used to denote seroprotection.^{16, 20, 21}

Descriptive statistics were used to evaluate demographics, subject characteristics and adverse events by study group. Univariate analyses were performed to test seroprotection and post-vaccination antibody titers between groups. Antibody titers were log-transformed for normality and GMTs were reported. Proportions of children with antibody titer $\geq 1:32$ were obtained and compared between groups. Categorical variables were compared using the Chi-square or Fisher exact tests, as appropriate. Continuous variables, including the primary outcome, were compared using the t test. To adjust for potential confounders and covariates, multiple linear regression analysis was carried out to evaluate the association between post-vaccination antibody titers and gestational age groups, after controlling for postnatal age at first dose of vaccine, gender, and weight at first vaccine. All the statistical tests were two-sided and the significance level was set at 0.05. Since pre- and post-titers were not compared with each other, serotypes were not compared to one another, and each year's values were considered separately, adjustments were not made for multiple comparisons. Analyses were performed with SAS (version 9.1, SAS Institute Inc, Cary, NC).

RESULTS

A total of 41 premature (9 in 2006–7, 32 in 2007–8) and 42 full-term (8 in 2006–7 and 34 in 2007–8) children were enrolled. All 17 subjects in 2006–7 had complete data. In 2007–8, however, post-vaccine titers were not available for 5 premature infants (1 received only one vaccination and 4 had no final blood draw) and for 9 full-term infants (6 had only one vaccination and 3 lacked the final blood sample), leaving 83% of infants enrolled during the two years with evaluable data. Since the vaccine components differed by year, data are presented separately for each year. Demographic and neonatal information (Table, Supplemental Digital Content 1) and vaccination characteristics (Table 1) are reported for children with post-vaccination titers available. Premature infants had mean gestational ages of 26.4 – 26.9 weeks at birth, while mean full-term infant gestational ages were 39.5 – 40.3 weeks. Sixty-nine percent of the premature infants had bronchopulmonary dysplasia, defined as supplemental oxygen administration at 36 weeks' postmenstrual age. The demographic characteristics were similar between subjects who had sera available and those who did not (data not shown). In 2006–7, postnatal age at first vaccine ranged from 6.0 to 15.1 months (with a median of 9.0 months and an interquartile range [IQR] of 6.5 to 10.3 months) in premature infants and 6.0 to 7.3 months (median 6.3 months, IQR 6.1 to 6.7 months) in full-term infants. In 2007–8, the age ranges were 6.0 to 16.8 months (median 11.2 months, IQR 6.6 to 13.0 months) in premature infants and 6.0 to 16.1 months (median 9.6 months, IQR 6.6 to 12.6 months) in full-term infants. Two subjects who were not receiving systemic glucocorticoids at enrollment received oral glucocorticoids at some point between the first vaccine dose and second blood draw.

Figure 1 shows the antibody GMT for each season. Premature infants had higher titers than full term infants to the H1 and B/Victoria vaccine components in the 2006–7 season and higher titers to the H1 component in the 2007–8 season. Table 2 shows the proportions of children in each year with antibody titers at or above the presumed protective titer of 1:32. A higher proportion of premature than full-term children had B/Victoria titers $\geq 1:32$ in the 2007–8 season. Multiple linear regressions were fitted after controlling for postnatal age at vaccination, weight at vaccination and gender, which differed between groups. After adjusting for these possible confounding variables, study group (PT vs. FT) no longer

showed a significant effect on titer for any vaccine component (Table 3). In addition, none of the potential confounding variables showed consistent effects on titers in the model.

Adverse events are detailed in Table, Supplemental Digital Content 2. The most common events were fever and/or acute respiratory illness. Two (5%) premature and 8 (19%) full-term infants had adverse events, primarily fever, likely to be vaccine-associated within 72 hours after vaccination. There were seven severe adverse events among premature infants, but none among full-term infants. No infant in either group had apnea, suffered a severe adverse event in the 72 hours following influenza immunization, or had medically-diagnosed influenza-like illness between the first immunization and the final blood draw.

DISCUSSION

Contrary to our hypothesis, premature infants 6–17 months chronological age did not have lower humoral immune responses to two doses of TIV than those of full-term infants of similar age. Infants in both groups experienced possible vaccine-related adverse events, most commonly fever. Premature, but not full-term, infants had hospitalizations during the study period, but no hospitalization appeared related to vaccination.

Influenza vaccine's ability to prevent infection is modest even in healthy, full-term children. The only influenza vaccines currently licensed in the U.S. for children younger than 2 years of age are trivalent, inactivated influenza vaccines (TIV), which contain 3 antigens (most commonly two influenza A antigens and one B antigen) each year. Antibody titers measured by HAI have been traditionally used to assess the *immunogenicity* (ability to induce adequate titers of antibody) of TIV because of their general correlation with protection against native influenza disease or intranasal viral challenge.^{22, 23} Although TIV may induce adequate titers of antibody to at least one of the vaccine antigens in up to 94% of children, including those as young as 6 months, as few as 35% respond adequately to all three antigens.^{20, 24} Estimates of TIV *efficacy* (ability to prevent disease in a controlled trial) in children range widely, from 12–100%, depending on the study, population, and match of vaccine strain to circulating strain.^{10, 25–28} A recent, multi-year study estimated the *effectiveness* (ability to prevent disease in a clinical setting) of full vaccination against laboratory-confirmed influenza at 86% and of partial vaccination at 73% among children 6–39 months old.²⁹ On the other hand, the effectiveness of TIV in preventing influenza-like illnesses has been estimated at about 23–25% among children who receive two doses of vaccine.^{13, 30, 31} In two years (2003–4, 2004–5) in which the vaccine was relatively poorly matched to the circulating strains, TIV effectiveness in preventing medical visits due to culture-confirmed influenza could not be demonstrated in children <5 years old.^{15, 32} Although TIV can produce adequate antibody responses in children as young as 6 weeks of age,^{33, 34} several studies have suggested that TIV effectiveness is low, approaching 0%, in children < 2 years of age.^{12–15}

Previous studies of influenza vaccines in premature infants have yielded conflicting results. Groothuis and colleagues examined the response of 15 previously unimmunized, 6-to-18-month old former premature infants with continued symptoms from BPD to 2 doses of TIV.¹⁶ Although greater than 90% of children developed acceptable rises in HAI antibody titer ($\geq 1:32$), premature infants had mean antibody titers to each of the three vaccine components about one-half those of 18 previously unimmunized, 6-to-18-month-old, healthy, full-term children. The premature infants also had influenza-specific T-cell proliferative responses about one-half those of full term children. Another group of 30 previously immunized, 6-to-48-month-old former premature infants, half of whom had continued symptoms from BPD and half of whom had recovered from BPD, also had lower T-cell proliferative responses after reimmunization than healthy full term children.¹⁶

In another study of 43 former premature infants, ages 9–44 months, with BPD, 17% (influenza B antigen) to 75% (influenza A/H3N2 antigen) of children achieved a 4-fold rise in titers following administration of TIV.¹⁷ More recently, 45 previously unimmunized former premature infants, ages 6–11 months, were reported to produce antibody responses to TIV comparable to those historically reported in full-term infants, but no concurrent full-term control group was included.¹⁸ Our data confirm the more recent findings in a group with concurrent control subjects.

Several baseline differences between the premature and full-term infant groups existed in this study. Including the potentially confounding factors of gender, postnatal age at vaccination and weight at vaccination in a multivariate regression analysis removed the significant association observed in univariate analysis between prematurity and higher titers to some vaccine components. This suggests that one or more confounding factors in the premature infants may have accounted for the higher titers observed among the premature infants. That is, while the titers were higher in the premature than the full-term group, this difference was likely to be due to a factor other than prematurity itself. The number of subjects is too small to permit adequately powered stratified analyses to explore this question further.

This study has limitations. Since influenza vaccine antigens varied between 2006–7 and 2007–8, we separately evaluated each year's antibody results. This limited the power of the comparisons, particularly for 2006–7. Nevertheless, the premature infants' antibody responses remained at least as high as those of full-term infants in both years. Although antibody response measured by HAI titer is generally related to protection against influenza infection, elevated HAI titers alone may not offer significant protection against influenza in young infants.^{12–15} The results of HAI titers among our premature infants may therefore be falsely reassuring. Although no infant experienced medically-diagnosed influenza during the study period, the mild elevation of B/Yamagata titers (an antigen not contained in the vaccine) in 2007–8 suggested that some infants may have experienced sub-clinical infection with influenza A or B. Both circulated during each of the 2 seasons and may have influenced the antibody titer results.

In summary, our study findings suggest that the current TIV vaccines are immunogenic and capable of generating measurable antibody responses among very premature infants. This strongly supports immunizing premature infants as currently recommended. However, whether antibody responses alone predict protection against influenza among young formerly premature infants remains to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Geometric mean titers (GMT) of influenza antibody

Titers (expressed as inverse GMT $\pm$ 95% confidence interval) to each of the three vaccine components and an unrelated B antigen (B/Yamagata) are shown for the 2006–7 (A) and 2007–8 (B) seasons. Sera were available from 9 preterm (PT) and 8 full-term (FT) pre- and post-vaccination for 2006–7, and 21 PT and 30 FT pre-vaccination and 27 PT and 25 FT post-vaccination for 2007–8. Post-vaccine GMT for 2006–7 for PT and FT infants, respectively, were H1: 1:513 v. 1:91; H3: 1:202 v. 1:118; B Victoria: 1:51 v. 1:10; B Yamagata: 1:4 v. 1:7. Post-vaccine GMT for 2007–8 for PT and FT infants, respectively, were H1: 1:363 v. 1:189; H3: 1:38 v. 1:22; B Victoria: 1:54 v. 1:27; B Yamagata: 1:13 v. 1:7. *P <0.05 by Student t test for PT to FT comparisons.

Table 1

Vaccination characteristics among subjects with post-vaccination titers available.

	2006-7			2007-8		
	PT group (n = 9)	FT group (n = 8)	P*	PT group (n = 27)	FT group (n = 25)	P*
Age at 1 st vaccination (mo $\pm$ SD)	9.0 $\pm$ 3.1	6.4 $\pm$ 0.5	0.03	10.4 $\pm$ 3.2	9.9 $\pm$ 3.4	0.54
Age (corrected for gestation) at 1 st vaccination (mo $\pm$ SD)	6.0 $\pm$ 3.0	6.5 $\pm$ 0.5	0.69	7.3 $\pm$ 3.3	9.7 $\pm$ 3.5	0.01
Weight at 1 st vaccine (kg $\pm$ SD)	7.0 $\pm$ 1.3	8.3 $\pm$ 0.7	0.02	7.3 $\pm$ 1.4	9.1 $\pm$ 1.6	<0.01
Received oral glucocorticoids during vaccine series (%)	1 (11%)	0	1.0	1 (4%)	0	1.0
Time from 1 st to 2 nd vaccine (wk $\pm$ SD)	4.2 $\pm$ 0.4	4.3 $\pm$ 0.4	0.77	5.9 $\pm$ 2.4	5.6 $\pm$ 1.9	0.54

PT = premature, FT = full-term, SD = standard deviation. Results are presented as mean $\pm$ SD or N (%).

* P value for PT vs. FT by t test, chi-square or Fisher exact test, as appropriate

Proportion of children with post-vaccine antibody titer $\geq 1:32$.

Hemagglutinin	2006-7			2007-8		
	PT group (n = 9)	FT group (n = 8)	P [†]	PT group (n = 27)	FT group (n = 25)	P [†]
H1	9 (100%)	7 (88%)	0.47	27 (100%)	24 (96%)	0.48
H3	9 (100%)	6 (75%)	0.21	20 (74%)	12 (48%)	0.05
B/Victoria	6 (67%)	3 (38%)	0.35	23 (85%)	15 (60%)	0.04
B/Yamagata *	0	0	--	8 (30%)	5 (20%)	0.42

[†]p for FT vs. PT by chi-square or Fisher exact test, as appropriate

Table 3

Outcome of multiple linear regressions for effect of preterm group (v. full-term group) on log 10 antibody titer.

Serotype	Estimated adjusted difference in log 10 titer (95% CI)	
	2006-7	2007-8
H1	0.87 (-0.39, 2.14)	0.35 (-0.02, 0.72)
H3	0.10 (-1.07, 1.28)	0.31 (-0.07, 0.69)
B/Victoria	0.83 (-0.25, 1.92)	0.44 (-0.04, 0.92)
B/Yamagata	-0.02 (-0.50, 0.46)	0.20 (-0.54, 0.93)

CI = confidence interval

Multiple linear regressions were fitted after controlling for postnatal age at vaccination, weight at vaccination and gender, none of which showed consistent effects on titers in the model. Positive numbers indicate higher titers in preterm group, and difference is considered statistically significant if 95% CI does not cross zero.