Pregnancy Modifies the Antibody Response to Trivalent Influenza Immunization

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We report the immunogenicity of trivalent influenza immunization in 29 pregnant women compared with 22 nonpregnant women. We obtained blood specimens on day 0 prior to 2011–2012 influenza vaccine administration and day 28 after immunization. Hemagglutination inhibition (HAI) geometric mean titers were similar before immunization but were significantly reduced by 40%–50% in pregnant women after immunization for influenza A/California (H1N1) (P = .027) and A/Perth(H3N2) (P = .037). Post-immunization HAI titers were similar between groups for influenza B/Brisbane (P = .390). The geometric mean ratio (fold increase) for influenza A(H1N1) was nonsignificantly reduced in pregnant participants (P = .089). The percentages of participants who seroconverted and achieved seroprotection were similar between groups.

Healthy pregnant women are at increased risk of morbidity and mortality from influenza virus infection, and seasonal influenza-related hospitalizations are increased in this population [1]. During 8 influenza seasons studied in the Tennessee Medicaid population, 5.1 per 1000 women with singleton pregnancies had respiratory disease hospitalizations [1]. During the 1918–1919 influenza pandemic, the case-fatality rate of hospitalized pregnant women with influenza was reported to be 30%–50% in the United States [1]. Similarly, the 2009 influenza A(H1N1) pandemic disproportionately affected pregnant women [2].

Despite this preliminary understanding of maternal clinical risk, the physiologic mechanisms that predispose young, healthy pregnant women to worse outcomes with influenza virus infection are not understood. Tolerance of the fetal allograft requires suppression of type 1 T-helper (Th1) cell-mediated activity with a parallel shift to Th2 activity [3]. Suppressed Th1 immunity likely impairs maternal response to infection with viruses, including influenza viruses. These immunologic changes may also lead to a decreased immune response to vaccines.

Influenza vaccine is recommended in pregnancy [4], and several studies have shown that antenatal influenza vaccine is highly effective in preventing clinical disease in both mothers and their infants [5]. A unique randomized trial in Bangladesh demonstrated a significant 63% reduction of laboratory-confirmed influenza among infants of mothers who received influenza vaccine, with a 36% reduction of flulike illnesses in the mothers [6, 7]. Other studies in the United States have confirmed these findings [8]. Reports from 23–48 years ago state that the antibody response to historical influenza vaccines are similar in pregnant and nonpregnant women [9, 10], but there are no reports comparing current influenza vaccines.

Recent analyses of monovalent pandemic 2009 influenza A (H1N1) vaccine in pregnant women reveal adequate immunogenicity after immunization [11–14], but to our knowledge, there are no reported studies of current trivalent seasonal influenza vaccine in pregnant women compared with nonpregnant women.

Despite data on the clinical efficacy of influenza vaccine in pregnant women and their infants, there is limited information on the details of the immunological responses to influenza immunization during pregnancy. The primary goal of this study is to describe how pregnancy modulates the immune response to influenza immunization. We conducted a prospective clinical study to investigate the antibody responses to influenza immunization, an influenza-related stimulus in pregnancy.

METHODS

This is a prospective comparative study of 51 pregnant and nonpregnant women aged 18–39 years at Cincinnati Children’s Hospital Medical Center. Women were eligible to participate if they were 18–39 years of age and had not yet received
a 2011–2012 influenza vaccine. Pregnancy was defined by maternal or physician report, with expected delivery on or after 1 November 2011. Women with diagnoses of cancer, with defects of immune response, receiving immunomodulatory therapy, or with other chronic diseases were excluded.

The 51 participants were enrolled from 3 October 2011 through 17 October 2011. We obtained informed consent at the time of enrollment. Participants answered questions about age, date of birth, race/ethnicity, medical insurance, and previous receipt of influenza vaccine. Pregnant participants answered the same questions plus additional questions about gravidity, parity, last menstrual period, estimated date, and method of calculating due date (last menstrual period versus ultrasound).

We obtained approximately 20 mL of blood by venipuncture and intramuscularly administered the 2011–2012 trivalent influenza immunization, Fluarix (lot no. AFLUA004BC; GlaxoSmithKline Biologicals). The 2010–2011 vaccine included antigens similar to influenza A/California/7/2009(H1N1), A/Perth/16/2009(H3N2), and B/Brisbane/60/2008. Participants were observed for 15 minutes after receipt of vaccine. Participants returned on day 28 (range, 24–31 days) after immunization for repeat blood draws. If the participant gave birth between visits, she was asked to provide the infant’s date of birth, birth weight, gestational age, and any history of fever (temperature, >100.4°F) since birth.

We used a hemagglutination inhibition (HAI) assay on day 0 (prevaccine) and day 28 (postvaccine) to assess antibody responses to influenza immunization. The analysis of the serum samples was performed by the Laboratory for Specialized Clinical Studies at Cincinnati Children’s Hospital Medical Center. This laboratory is certified by the Clinical Laboratory Improvement Amendments and the College of American Pathologists. Serum samples were first assessed for antibody to each of the 3 components of the vaccine by HAI assay using standard methods [6].

The original sample size calculations were based on a primary analysis of cytokine levels in maternal serum samples. A sample size of 20 pregnant women provided 80% power to detect at least a 30% difference in cytokine levels between pregnant and nonpregnant women, based on a Student t test with a 2-sided significance level of P < .05.

We used a 2-sample t test to compare geometric mean titers (GMTs) of HAI titers between pregnant and nonpregnant women before (day 0) and after influenza immunization (day 28). We also calculated the geometric mean ratio (GMR), or the fold increase in HAI titers before and after immunization, and compared these in pregnant and nonpregnant women using a paired t test. We calculated the percentage of seroconverted (≥4-fold increase in titer) participants in each group. We also calculated the percentage of seroprotected (HAI titer, ≥1:40) participants in each group and compared seroconversion and seroprotection percentages between pregnant and nonpregnant women using the χ2 test. P values of <.05 (2-tailed) were considered statistically significant, and comparisons were made without adjustments for baseline differences.

The protocol was approved by the institutional review board of Cincinnati Children’s Hospital Medical Center. Informed consent was obtained from participants, and human experimentation guidelines of the US Department of Health and Human Services and those of Cincinnati Children’s Hospital Medical Center were followed in the conduct of this research.

RESULTS

A total of 52 participants were enrolled in the study between 3 October 2011 and 17 October 2011. One participant could not provide the day 28 specimen due to premature delivery of her infant. The subject characteristics are shown in Table 1. Of 51 participants, 29 were pregnant at the time of influenza immunization (3 in the first trimester, 18 in the second, and 8 in the third). The mean age was significantly higher for pregnant participants (P = .018), and there were significantly more Caucasian women in the pregnant group (P = .036). All participants were privately insured. Most participants reported receiving a

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<th>Table 1. Characteristics of Participants</th>
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Data are no. (%) of women, unless otherwise specified.
seasonal influenza vaccine in 2010, although the percentages receiving influenza vaccine in preceding years were decreased. The numbers of participants receiving any previous influenza immunizations were similar between groups (Table 1).

HAI assay results are presented in Table 2. The preimmunization GMTs were similar in pregnant and nonpregnant women for each of the 3 influenza vaccine antigens. Postimmunization HAI GMTs were significantly reduced by 40%–50% in pregnant women for A/California(H1N1) (P = .027) and A/Perth(H3N2) (P = .37).

The GMR was reduced nonsignificantly in the pregnant participants for A/California(H1N1) (P = .089) (Supplementary Figure 1). The percentage of participants who seroconverted and the percentage with seroprotection were similar between pregnant and nonpregnant women. The percentage of seroprotected women was >90% in both pregnant and nonpregnant women for A/California(H1N1) and A/Perth(H3N2) viruses. However, only 58.62% of pregnant and 66.18% of nonpregnant women demonstrated seroprotection to B/Brisbane.

**DISCUSSION**

This is a unique report comparing immune responses to current trivalent influenza vaccine in pregnant and nonpregnant women. We demonstrate a significantly decreased GMT and nonsignificantly decreased GMR (fold increase) to influenza A antigens after influenza immunization in pregnant women, although overall seroconversion rates and seroprotection rates were comparable in pregnant and nonpregnant groups. Approximately 1 month after influenza immunization, >90% of women demonstrated seroprotective titers to both A/California(H1N1) and A/Perth(H3N2) antigens. However, seroprotection against B/Brisbane antigen remained <60% in pregnant women and <70% in nonpregnant women at 28 days after immunization. These results suggest that pregnant women exhibit a decreased geometric mean antibody response to influenza A vaccination antigens but high proportions achieve seroprotection.

There are several possible explanations for the discordance between lower GMTs in pregnant women with nonsignificant differences in the proportion with seroprotective HAI levels. We noted significant differences in age and race/ethnicity between pregnant and nonpregnant groups, which could be associated with differences in immune response. However, the preimmunization GMTs and influenza A(H1N1) vaccination history were similar between groups, suggesting the immunogenicity variation may be due to pregnancy rather than a 2-year difference in mean age or a difference in the race/ethnicity composition of subjects. We are not aware of trivalent influenza vaccine immunogenicity differences associated with race or small differences in age. These preliminary data will be evaluated with additional subjects and current vaccine antigens to assess these differences. Additional studies are needed of subpopulations and their response to influenza vaccine antigens, as well as analyses of additional immunologic factors.
To our knowledge, this is the first report of the immunogenicity of a current influenza vaccine in pregnancy for 3 influenza vaccine antigens and with a direct comparison with nonpregnant women. These results are similar to findings in previous studies that analyzed the immunogenicity of influenza A(H1N1) vaccine and demonstrated that high proportions of pregnant women developed seroprotective antibody levels [11–14]. Ohtfuji et al reported a lower seroprotective antibody response to influenza A(H1N1) vaccine in pregnant women who had received prior seasonal vaccination [11]. Jackson et al showed 93% and 97% seroprotection after vaccination of pregnant women with influenza A(H1N1) vaccine containing 25 μg and 49 μg of hemagglutinin, respectively [12]. Tsatsaris et al showed a seroprotective response in 98% of pregnant women and in 95% of their infants after antenatal influenza A (H1N1) vaccine [13]. Fisher et al showed similar GMTs to influenza A(H1N1) vaccine and influenza virus infection in pregnancy [14]. A recent analysis of trivalent inactivated influenza vaccination in pregnant and postpartum women revealed no statistically significant differences between seroconversion rates by trimester or in the immediately postpartum period, but this study did not include a comparison group of nonpregnant, non-postpartum women [15]. None of these recent studies have compared immunologic responses between pregnant and nonpregnant women. Our approach provides a broader description of the antibody response in pregnancy, utilizing a comparison as well as a more comprehensive vaccine antigen panel.

Possible limitations of this study include the small sample size, the potential for confounding, and the lack of vaccine efficacy data. Although this analysis was useful for comparing pregnant and nonpregnant women, we plan to conduct larger studies to assess differences in subgroups. Because influenza vaccine antigens vary by year, these data from one vaccine formulation may not describe responses of pregnant women to other influenza antigens.

These data will be useful in understanding more fully the effects of pregnancy on the immunogenicity of influenza antigens. They may also provide insights into the effects of natural influenza virus infection. Data from this study will guide further studies, including analyses of subject subgroups and gene transcription analyses. Additionally, maternal immunization studies may provide insights into the mechanism of fetal effects during antenatal influenza virus infection.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copiededit. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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