Early Changes in Interferon Gene Expression and Antibody Responses Following Influenza Vaccination in Pregnant Women

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Background. Influenza immunization during pregnancy provides protection to the mother and the infant. Studies in adults and children with inactivated influenza vaccine have identified changes in immune gene expression that were correlated with antibody responses. The current study was performed to define baseline blood transcriptional profiles and changes induced by inactivated influenza vaccine in pregnant women and to identify correlates with antibody responses.

Methods. Pregnant women were immunized with inactivated influenza vaccine during the 2013–2014 and 2014–2015 seasons. Blood samples were collected on day 0 (before vaccination) and on days 1 and 7 after vaccination for transcriptional profile analyses, and on days 0 and 30, along with delivery and cord blood samples, to measure antibody titers.

Results. Transcriptional analysis demonstrated overexpression of interferon-stimulated genes (ISGs) on day 1 and of plasma cell genes on day 7. Prevaccination ISG expression and ISGs overexpressed on day 1 were significantly correlated with increased H3N2, B Yamagata, and B Victoria antibody titers. Plasma cell gene expression on day 7 was correlated with increased B Yamagata and B Victoria antibody titers. Compared with women who were vaccinated during the previous influenza season, those who were not showed more frequent significant correlations between ISGs and antibody titers.

Conclusions. Influenza vaccination in pregnant women resulted in enhanced expression of ISGs and plasma cell genes correlated with antibody responses.

Keywords. influenza vaccine; immunization; maternal immunization; antibodies; Interferon-stimulated genes (ISGs).

Pregnant women are at increased risk for influenza-associated illness and death, as well as for serious fetal complications, such as preterm birth, fetal growth restriction, and fetal demise [1, 2]. The Advisory Committee on Immunization Practices and the American College of Obstetricians and Gynecologists recommend seasonal influenza vaccination to all pregnant women, or women who will become pregnant during influenza season [3, 4]. Because no influenza vaccine is licensed for women, or women who will become pregnant during influenza season, this population relies on maternal antibodies for protection, hence the importance of vaccination during pregnancy.

Inactivated influenza vaccination has been proved safe for both the pregnant women and their fetuses [5, 6], and it provides effective transfer of protective antibodies to newborns [7–9]. It has also been shown that these antibodies are capable of protecting newborns and infants in the first months after birth against severe influenza infection and consequences including pneumonia, hospitalization, and death [10–14]. Studies, however, have shown that influenza vaccination in the previous season affects antibody responses to subsequent vaccination, and there are concerns that this may compromise neonatal protection. A recent study showed that despite lower antibody titers after influenza vaccination in women who had been vaccinated the previous year, the antibody titers measured in cord blood were protective [15].

Studies have applied transcriptome analyses to study the role of innate and adaptive immune genes in response to influenza vaccination in different age groups and patient populations to identify the potential mechanisms and specific immune cells associated with protective immune responses. In the elderly, adults, and children influenza vaccine has been found to be associated with activation of interferon-stimulated genes (ISGs).
early after vaccination (1–7 days), which are correlated with antibody responses [16–22]. Because pregnancy significantly modulates immune responses [23–25] and has been associated with reduced ability to effectively respond to certain viral pathogens causing respiratory infections [26], we investigated whether influenza immunization in pregnant women would induce effective activation of innate immune responses, specifically interferon signaling, as observed in other populations. In addition, we assessed whether those changes were correlated with antibody responses, and whether receipt of seasonal influenza vaccine the preceding year affected the transcriptional immune profiles measured before and after administration of the current year’s vaccine.

METHODS

Study Design and Participants

This was a prospective cohort of previously healthy pregnant women 18–42 years of age, <29 weeks pregnant, with plans to deliver at the Ohio State University Wexner Medical Center (OSUWMC). They were enrolled from October 2013 to April 2015 and immunized with quadrivalent inactivated influenza vaccine. Peripheral blood samples were collected on day 0 (before vaccination) and days 1 and 7 (after vaccination) for transcriptional profile analysis, and on day 0, day 30, and at delivery to measure maternal antibody titers. Cord blood samples were also collected to measure the antibody transfer to the neonates. One hundred four women were enrolled in the parent study; 5 withdrew before completion. Forty-five women had samples available for all the time points evaluated; as such, they were selected as a convenient sample for the present study. Exclusion criteria included current or past major immunological disorder conditions, such as human immunodeficiency virus, systemic lupus erythematosus, and cancer. One woman was excluded from the analysis owing to the high ISG expression at baseline (Supplementary Figure 1), and 2 others did not have cord blood samples obtained; accordingly, 44 women were included in the entire analysis and 42 for cord blood titers analysis.

This study used the vaccine formulation from the 2013–2014 and 2014–2015 seasons containing A/California/7/2009 (H1N1)pdm09-like virus, A/Texas/50/2012 (H3N2)-like virus, B/Massachusetts/2/2012-like virus (Yamagata lineage), and B/ Brisbane/60/2008-like virus (Victoria lineage). For women vaccinated in 2014–2015, those vaccinated the previous year received the same vaccine. For women vaccinated in 2013–2014, those vaccinated the previous year received Trivalent inactivated influenza vaccine, which included the same H1N1 and H3N2 strains and a different B strain, B/Wisconsin/1/2010-like virus [15]. The study was approved by the OSUWMC Biomedical Institutional Review Board (no. IRB2008H0260) and was classified as a level 1 risk clinical study (no greater than minimal risk; pursuant under 45 CFR 46.404; and 21 CFR 50.51). Written informed consent was obtained from all participants before enrollment, in accordance with OSUWMC guidelines. Complete data on antibody responses among women in the parent study cohort were reported elsewhere [15].

Serology

Serum samples were collected at 3 time points from pregnant women (on days 0 and 30 and at delivery) and from cord blood. The hemagglutination inhibition (HI) assay was performed as described elsewhere [27]. The HI titer was defined as the reciprocal of the highest dilution of serum that inhibits red blood cell hemagglutination. Seroconversion was based on the following criteria: a 4-fold increase in antibody titers between the prevaccination and the convalescent-phase serum samples or an increase in antibody titers from <10 to ≥40 for the prevaccination and convalescent-phase serum samples. Seroprotection was defined as an HI titer ≥40 after vaccination [28].

Transcriptional Profiles and Statistical Analysis

Whole-blood samples (1 mL) were collected in Tempus tubes (Applied Biosystems) and stored at −80°C. After RNA was processed [29, 30] and hybridized into Illumina Human HT-12 v4 BeadChips (47.323 probes), it was scanned on the Illumina Beadstation 500. Illumina GenomeStudio software (version 2) was used for data preprocessing (background subtraction and normalizations). The limma package in R software (Version 3.48) was used for downstream analyses [31, 32]. Transcripts were first selected if they were present in ≥10% of all samples. Next, raw expression values <10 were set to 10, and the data were log transformed. Differentially expressed genes between the groups were identified using the limma package in R with moderated t tests and Benjamini-Hochberg correction for multiple comparisons, followed by a 1.25-fold change in gene expression. The false discovery rate was set at <0.01. To assess the function of the differentially expressed genes we used a modular transcriptional repertoire framework as described elsewhere [33, 34]. Data are deposited in the National Center for Biotechnology Information Gene Expression Omnibus (accession no. GSE166545).

Demographic data were statistically analyzed using Graph Pad Prism software (version 8.20). Quantitative data were described as medians with interquartile ranges, and qualitative data as percentages. Correlations with antibody titers were performed using the Spearman correlation coefficient in SigmaPlot software. Figures for correlation analysis were performed using R software (version 14) (R Foundation for Statistical Computing).

RESULTS

Antibody Responses to Influenza Vaccine

HI antibody titers for each of the 4 influenza strains contained in the vaccine were measured in serum samples from 44 women
(demographic data summarized in Table 1) at days 0 and 30, at delivery, and in cord blood. We were not able to obtain cord blood samples in 2 women, so we included 42 women in the analysis. We observed significantly higher antibody titers at day 30 than at day 0 and at delivery for the 4 strains of the vaccine, H1N1, H3N2, B Yamagata, and B Victoria. Baseline titers are summarized in Supplementary Table 1. Cord blood antibody titers were also significantly higher compared with maternal titers at delivery (P < .001; Friedman and Dunn multiple comparisons tests) (Supplementary Table 2).

Seropositive rates at day 30 were 83%, 88%, 76%, and 64% for H1N1, H3N2, B Yamagata, and B Victoria, respectively. Cord blood seropositive rates were 100% for H1N1, H3N2, and B Yamagata, and 97% for B Victoria. Seroconversion rates were 31%, 45%, 33%, and 47% for H1N1, H3N2, B Yamagata, and B Victoria, respectively (Supplementary Table 2).

**Antibody Responses Based on Previous Season Vaccination**

We compared the antibody responses in pregnant women who received influenza vaccine the previous season (n = 29 [65.9%]) with those in women who did not (n = 15 [34.1%]). Women vaccinated the previous year had seroprotection rates at day 30 of 77% for H1N1 and H3N2, 70% for B Yamagata, and 63% for B Victoria. Those not vaccinated the previous year had seroprotection rates at day 30 of 80% for H1N1, 93% for H3N2, 73% for B Yamagata, and 60% for B Victoria.

Women who were not vaccinated the previous season had significantly higher fold change of HI titers for H1N1, H3N2, and B Yamagata strains on day 30 after vaccination compared with day 0 (P < .01; Kruskal-Wallis and Dunn multiple comparisons tests) (Figure 1A). They also had significantly higher fold changes of HI titers for H1N1 and B Yamagata when we compared delivery with day 0 (P < .01; Kruskal-Wallis and Dunn multiple comparisons tests) (Figure 1B). We did not observe any significant differences in cord blood titers according to vaccination in the previous season (Figure 1C).

**Significant Early Changes in Transcriptional Profiles After Vaccination**

We analyzed whole-blood gene expression profiles in 44 pregnant women on day 0 (before vaccination) and on days 1 and 7. Samples obtained on day 0 were used as the reference for comparisons.

Statistical group comparisons yielded 1217 differentially expressed transcripts among the 3 time points evaluated (P < .01; moderated paired t test and Benjamini-Hochberg multiple test correction; fold change >1.25), with marked changes observed on day 1 (Figure 2A). Of those, 51.3% of genes were underexpressed, and 48.7% overexpressed. To characterize the immune function of the differentially expressed genes, we applied the modular expression analysis tool [34]. Gene expression levels were compared on a module-by-module basis between samples obtained on days 1 and 7 in relation to day 0 samples that served as the reference. For each module, the percentage of genes significantly expressed (P < .05; moderated paired t test) are shown in the modular map. On day 1, we observed marked overexpression of the 3 ISG-related modules (M1.2, M3.4, and M5.12) and also increased expression of the monocyte (M4.14), inflammation (M4.6), and antiviral response (M8.59) modules. On the other hand, there was underexpression of the T-cell (M4.1 and M4.15), cytotoxic/natural killer cell (M3.6)...

### Table 1. Demographic Characteristics of the 44 Women Included in the Analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women, No. (%)a</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 44)</td>
</tr>
<tr>
<td>Age, median (IQR), y</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>31 (70.5)</td>
</tr>
<tr>
<td>Black</td>
<td>10 (22.7)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>Non-Hispanic ethnicity</td>
<td>44 (100)</td>
</tr>
<tr>
<td>Comorbid conditionsb</td>
<td>27 (61)</td>
</tr>
<tr>
<td>GA, median (IQR), wk</td>
<td></td>
</tr>
<tr>
<td>Delivery route and timing</td>
<td></td>
</tr>
<tr>
<td>SVD</td>
<td>32 (72.7)</td>
</tr>
<tr>
<td>Cesarean</td>
<td>12 (27.3)</td>
</tr>
<tr>
<td>Term</td>
<td>40 (90.9)</td>
</tr>
<tr>
<td>Preterm</td>
<td>4 (9.1)</td>
</tr>
</tbody>
</table>

Abbreviations: GA, gestational age; IQR, interquartile range; NPV, no prior vaccine (no receipt of influenza vaccine in the previous season); PV, prior vaccine (receipt of influenza vaccine in the previous season); SVD, spontaneous vaginal delivery.

aData represent no. (%) of women unless otherwise specified.

bComorbid conditions included obesity, preeclampsia, hypertension, diabetes, depression, thyroid disorder, hepatitis C infection, and active herpes simplex virus infection.
and B-cell (M4.10) modules. On day 7, the plasma cell module (M4.11) was markedly overexpressed, and the B-cell module (M4.10) that was underexpressed on day 1 was moderately expressed (Figure 2B).

**Effects of Prior Influenza Vaccination on Transcriptional Profiles**

Because women not vaccinated the previous season had significantly higher increases in antibody titers, we performed a comparative transcriptional analysis based on previous season vaccination history. For this purpose and to have a balanced number of women in both groups, we included all 15 women who were not vaccinated the previous season and 14 randomly selected women who were vaccinated the previous season. We compared the gene expression changes observed on day 1 in these groups of women, since this was the time point when the most significant changes were observed in the analysis of the complete study cohort. Statistical group comparisons yielded 501 differentially expressed transcripts between the 2 time points (day 1 vs day 0) for both groups, and 119 of these transcripts were shared by them ($P < .01$; moderated paired t test).

**Figure 1.** Antibody responses based on previous season influenza vaccination, comparing women who had received an influenza vaccine in the previous season (PV) and those who had not (NPV). A, Fold change in hemagglutination inhibition (HI) titers at day 30 (compared with day 0 [before vaccination]) for each strain of the vaccine. B, Fold change in HI titers at delivery (compared with day 0). C, Cord blood HI titers. Red represents H1N1 strain; green, H3N2 strain; blue, B Yamagata strain; purple, B Victoria strain. Bars represent means with standard deviations. *$P < .01$ (Kruskal-Wallis and Dunn multiple comparisons tests).

![Figure 1](https://academic.oup.com/jid/article/225/2/341/6312681)

**Figure 2.** Transcriptional profile and modular expression analysis before and after influenza vaccination. A, Transcriptional profiles representing expression patterns before (day 0) and after (days 1 and 7) vaccination. Statistical comparison ($P < .01$; moderated paired t test and Benjamini-Hochberg multiple test correction; fold change [FC] > 1.25) between the time points yielded 1217 differentially expressed genes. Transcripts were organized by hierarchical clustering, where each row represents a single transcript and each column an individual woman. Normalized expression levels are indicated by red (overexpression) or blue (underexpression), compared with the median expression at baseline or day 0 (yellow). B, Modular expression analysis at day 1 after vaccination showed significant overexpression of interferon-stimulated gene (M1.2, M3.4, and M5.12) and monocyte (M4.14) modules and significant underexpression of T-cell–related modules (M4.1 and M4.15). At day 7 after vaccination, B cell (M4.10) and plasma cell (M4.11) modules were overexpressed as well. Each time point is compared with day 0. The color intensity of the spots (and the numbers displayed within the spots) represent the percentage of significantly overexpressed genes (red), underexpressed genes (blue), or no differences (white) compared with baseline (day 0) ($P < .05$; moderated paired t test). Abbreviation: NK, natural killer.
and Benjamini-Hochberg multiple test correction) (Figure 3A and 3B). Modular analysis showed that the 3 ISG modules (M1.2, M3.4, and M5.12) were markedly overexpressed and both T-cell modules (M4.1 and M4.15) were markedly underexpressed in the 2 groups of women. Other modules, such as the monocyte (M4.14) and antiviral response (M8.59) modules (overexpressed) and the B cell (M4.10) and cytotoxic/natural killer cell (M3.6) modules (underexpressed) showed similar patterns of expression in both groups. However, the plasma cell module (M4.11) showed moderate overexpression only in the group of women who were vaccinated the previous season. (Figure 3C).

Correlations Between Gene Expression and Antibody Production
On the basis of previous observations in other vaccinated populations [16, 18], we examined the correlations between ISGs and plasma cell gene expression and antibody production. For these analyses, we correlated the fold increase in HI titers at day 30 from all the women included in the study with the top 20 overexpressed ISGs on day 1, and with the top overexpressed plasma cell genes on day 7. These 2 different time points (days 1 and 7) were selected based on the initial findings on gene expression changes shown in Figure 2. There were moderate but significant correlations between several ISGs, including IFIT3, IFI44L, OAS3, and CXCL10, at day 1 and fold change in H3N2, B Yamanagata, and B Victoria titers \((r = 0.3–0.5; P < .05)\) (Figure 4A). There were also significant correlations between the plasma cell gene CD38 at day 7 and fold change of H1N1, H3N2, B Yamanagata, and B Victoria titers, as well as between several other plasma cell genes and increased antibody titers against both B strains \((r = 0.3–0.5; P < .05)\) (Figure 4B). Among these genes, several were related to immunoglobulin function, such as IGI, IGGV5, IGHG2, and IGGV1.

We also examined correlations between ISG expression measured in maternal samples on day 1 (total number of interferon-related genes \(n = 161\) included in the 3 modules M1.2, M3.4, and M5.12 and the top 10 overexpressed ISGs) with cord blood HI titers and did not find statistical significance. On the other hand, when we assessed the associations between plasma cell gene expression and cord blood HI titers in maternal samples at day 7, we identified a significant correlation between H1N1 titers and the gene IGK2-30 \((r = 0.37; P < .01)\).

Finally, we investigated whether the decline in antibody titers in maternal serum between day 30 and delivery were correlated with gene expression patterns. We identified significant correlations between 8 of the 10 top overexpressed ISGs on day 1 and fold decline in B Victoria titers \((r = 0.31–0.45; P < .05)\) (Supplementary Table 3), and significant correlation between
the plasma cell gene CAMK1G, which was overexpressed on day 7, and fold decline in B Victoria titers ($r = 0.38; P < .05$).

**Prevaccination ISG Expression and Antibody Responses**

We also sought to identify baseline prevaccination markers associated with protection, by correlating gene expression on day 0 with fold change in HI titers. We observed significant correlations between expression of ISGs, such as IFIT3, RSAD2, and OAS3, on day 0 and fold changes in antibody titers for H3N2, B Y amagata, and B Victoria ($r = 0.3–0.5; P < .05$) (Figure 4C).

Next, we assessed whether day 0 gene expression was correlated with seroconversion (defined by a 4-fold increase in antibody titers from day 0 to day 30). We observed significantly higher median expression of the 161 ISGs at day 0 in women who seroconverted for the B Victoria strain compared with women who did not ($P < .05$; Mann-Whitney test) (Figure 5A). Likewise, women who seroconverted to the B Victoria strain had significantly higher median expression of the 3 ISG modules at day 0 (M1.2, M3.4, and M5.12; $P < .05$; Mann-Whitney test) (Figure 5B), as well as the top 10 differentially expressed ISGs included in these modules (SERPING1, BATF2, IFIT3, IFIT44L, RSAD2, GBP6, GBP5, GBP1, IFIT3, and OAS3), compared with baseline ISG expression in women who did not seroconvert ($P < .05$; Mann-Whitney test) (Figure 5C). When analyzing the other vaccine strains, at day 0, women who seroconverted to the B Yamagata had significantly higher median expression of 2 of the top 10 differentially expressed ISGs, IFIT3 and OAS3, compared with baseline ISG expression of women who did not seroconvert ($P < .05$; Mann-Whitney test) (Figure 5C). There were no significant differences in baseline ISG expression and seroconversion with H1N1 and H3N2 strains.

**Effects of Previous Season Vaccination on Correlation Between Gene Expression and Antibody Titers**

We then investigated whether vaccination in the previous season affected correlations between ISGs and plasma cell genes and fold increase in antibody titers. First, we examined day 0 ISG expression in women who received the influenza vaccine in the previous season. We found significant correlations between 5 (SERPING1, IFIT3, GBP6, GBP1, and OAS3) of the top 10 overexpressed genes previously mentioned and fold changes in B Victoria antibody titers ($r = 0.4–0.48; P < .05$) (Figure 6A). Among women not vaccinated in the prior year, we observed significant correlations between the genes GBP6 and H1N1 fold increase in antibody ($r = 0.6; P < .05$) and between the genes BATF2, IFIT3, and

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**Figure 4.** Correlations between gene expression and increase in antibody titers after vaccination. **A,** Correlation between overexpression of interferon-stimulated genes (ISGs) at day 1 after vaccination and increases in antibody titers at day 30. **B,** Correlation overexpression of individual plasma cell genes at day 7 after vaccination and increases in antibody titers at day 30. **C,** Correlation between baseline (day 0) median individual ISG expression and fold change in hemagglutination inhibition titers at day 30. Yellow represents no significant correlations, and positive significant correlations ($P < .05$) are displayed on a red-orange scale, where the intensity of the color represents a higher Spearman correlation coefficient ($r$).
GBP6 and H3N2 fold increase in antibody titers (r = 0.53–0.55; P < .05) (Figure 6D). When considering day 1 ISG expression in women who received the influenza vaccine in the previous season, we did not observe any significant correlations (Figure 6B); while among those not previously vaccinated, the expression of OAS3 was significantly correlated...
Figure 6. Impact of previous season vaccination on correlation between gene expression and antibody titers. A–C, Median expression of individual interferon-stimulated genes (ISGs) found to be overexpressed at baseline (day 0) (A) or at day 1 (B) and of individual plasma cell genes (C), by increases in antibody titers at day 30 in study participants who received an influenza vaccine in the previous season. D–F, Median expression of individuals ISGs overexpressed at baseline (day 0) (D) or day 1 after vaccination (E) and of individual plasma cell genes (F), by increases in antibody titers at day 30 in women who did not receive an influenza vaccine in the previous season. Yellow represents no significant correlations, and positive significant correlations ($P < .05$) are displayed on a red-orange scale, where the intensity of the color represents a higher Spearman correlation coefficient ($r$).
with fold changes in H1N1 and H3N2 titers ($r = 0.58–0.61$; $P < .05$) (Figure 6E).

We performed similar analysis with plasma cell gene expression on day 7. Women who received influenza vaccine in the previous season showed significant correlations between 6 of the 20 genes included in the plasma cell module and fold increases in H3N2 titers ($r = 0.39–0.78$; $P < .01$), and between 10 of the 20 genes and fold increases in B Yamagata and B Victoria titers ($r = 0.53–0.83$; $P < .01$) (Figure 6C). On the other hand, women who did not receive the vaccine the previous season showed significant correlations between the genes CAMK1G and IGKV6-21 and fold increases in B Yamagata titers ($r = 0.55–0.59$; $P < .05$), and between the gene CAMK1G and fold increases in B Victoria titers ($r = 0.56$; $P < .05$) (Figure 6F).

**DISCUSSION**

The current study demonstrates that influenza vaccination elicits significant changes in expression of ISGs and plasma cell genes at 1 and 7 days after vaccination, respectively. These changes were correlated with antibody responses. In addition, baseline expression of ISGs was significantly correlated with antibody responses against influenza B strains.

Systems biology approaches have been used to define the molecular mechanisms of influenza vaccine immunity in different populations [16, 18, 19]. Transcriptome analysis to assess immune responses to influenza vaccine in pregnant women demonstrated early activation of ISGs and plasma cell–related transcripts after vaccination, suggesting that both innate and adaptive immune responses are readily stimulated by the vaccine to mount the immune response required for antibody production. These transcriptional immune responses are similar to those described in other populations [16–22].

These analyses demonstrate significant correlations between expression of specific immune genes and antibody responses. As in studies in other populations, we identified significant correlations between early postvaccination changes in ISG expression on day 1 and plasma cell genes on day 7 and antibody responses. In addition, we also observed significant correlations between baseline ISG expression, before vaccination, and antibody responses.

These significant correlations of ISG expression on days 0 and 1 with increased antibody titers confirms the role of this important component of the immune system in orchestrating influenza-vaccine induced protection, with activation of genes such as IFIT1, IFIT3, IFI44L, RSAD2, GBP1, and OAS3, which are interferon-induced antiviral proteins and act as inhibitors of cell migration, proliferation and signaling, as well as of viral replication.

When analyzing the correlations between gene expression and antibody responses overall, there were more frequent significant correlations with antibody responses to the B than to the A influenza strains. At this point, it is unclear whether these results reflect true differences in the immune pathways induced by the different vaccine strains. On other hand, this observation could be related to the lower baseline antibody titers for the B strains and subsequent greater vaccine antibody responses, which permitted an easier demonstration of those correlations. Likewise, we observed more significant correlations in women not vaccinated in the previous year, who also had greater fold changes in antibody responses that facilitated the recognition of the correlations.

A previously reported finding, which is not yet understood, is the underexpression of T-cell–related genes on day 1. Similar observations were reported on earlier studies of influenza vaccine in children and adults [18, 19]. Looking closely at the specific genes, we observed underexpression of the genes TCF7, CD40LG, CD3E, and CD27, all associated with T-cell differentiation and proliferation. The gene CD40LG is also involved in mediating B-cell proliferation and in immunoglobulin class switching. This observation will require future studies analyzing sorted cells and/or incorporating higher-resolution assays, such as single-cell sequencing, to elucidate its significance, especially considering the key role that helper CD4 T cells play in the adaptive immune response to influenza vaccine, stimulating B cells to produce antibodies [35].

Besides the currently available vaccines licensed for use in pregnant women, new maternal vaccines are being designed to prevent other severe infectious diseases in neonates and infants. Respiratory syncytial virus is the leading cause of viral lower respiratory tract infections in infants [36–39] and vaccines targeting infants and pregnant women are currently in clinical trials [40, 41]. Group B Streptococcus maternal immunization is also being targeted, because this bacterium is a common cause of neonatal sepsis [42]. Another candidate is a cytomegalovirus vaccine, owing to a higher risk of congenital infection when primary infection occurs during pregnancy [43]. More recently, in the context of the coronavirus disease 2019 pandemic, initial data suggest that vaccination of pregnant women is safe and can provide antibodies to protect newborns [44].

The current study has several limitations. First, vaccination history was based on self-report rather than medical record. We were able to confirm history of vaccination only in the previous year, and we compared baseline antibody titers between women who were vaccinated the previous year and those who were not. In addition, although we showed effective transfer of antibodies against influenza to the neonate through measurement in the cord blood, rates of clinically confirmed influenza infection and maternal antibody persistence in the infants were not examined and deserve further longitudinal studies.

In summary, we identified gene expression profiles at baseline (before vaccination) and early after vaccination that correlated with antibody responses in pregnant women vaccinated for influenza. Because the application of systems biology is becoming a cornerstone to study vaccine functionality, we believe...
this study provides additional insights into the mechanisms by which influenza vaccine provides immune protection in this population. Furthermore, it highlights the value of applying these analytical tools in this understudied population, suggesting that these approaches may be useful to help design and selecting improved and more effective maternal vaccines and should be incorporated more frequently into clinical studies.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
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Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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