Prolonged activation of innate antiviral gene signature after childbirth is determined by *IFNL3* genotype

Aryn A. Price[a,b], Dana Tedesco[a,b], Mona R. Prasad[c], Kimberly A. Workowski[d], Christopher M. Walker[e,f], Mehul S. Suthar[a,b,g], Jonathan R. Honegger[e,f,1], and Arash Grakoui[a,b,h,1]

[a]Emory Vaccine Center, Division of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30329; [b]Yerkes National Primate Research Center, Emory University, Atlanta, GA 30329; [c]Department of Obstetrics and Gynecology, The Ohio State University School of Medicine, Columbus, OH 43205; [d]Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322; [e]The Center for Vaccines and Immunity, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205; [f]Department of Pediatrics, The Ohio State University School of Medicine, Columbus, OH, 43205; [g]Department of Pediatrics and Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, GA 30322; and [h]Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30329

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Maternal innate and adaptive immune responses are modulated during pregnancy to concurrently defend against infection and tolerate the semiallogeneic fetus. The restoration of these systems after childbirth is poorly understood. We reasoned that enhanced innate immune activation may extend beyond gestation while adaptive immunity recovers. To test this hypothesis, the transcriptional profiles of total peripheral blood mononuclear cells following delivery in healthy women were compared with those of nonpregnant control subjects. Interestingly, interferon-stimulated genes (ISGs) encoding proteins such as IFIT1, IFIT2, and IFIT3, as well as signaling proteins such as STAT1, STAT2, and MAVS, were enriched postpartum. Antiviral genes were primarily expressed in CD14$^+$ cells and could be stratified according to genetic variation at the interferon-λ3 gene (*IFNL3*, also named IL28B) SNP rs12979860. Antiviral gene expression was sustained beyond 6 mo following delivery in mothers with a CT or TT genotype, but resembled baseline nonpregnant control levels following delivery in mothers with a CC genotype. CT and TT *IFNL3* genotypes have been associated with persistent elevated ISG expression in individuals chronically infected with hepatitis C virus. Together, these data suggest that postpartum, the normalization of the physiological rheostat controlling IFN signaling depends on *IFNL3* genotype.

postpartum | CD14 | interferon | innate immunity | antiviral genes

Maternal immunoregulatory adaptations such as the expansion of regulatory T cells (T_{regs}) with potent immunosuppressive capacity serve to maintain fetal tolerance during pregnancy (1, 2). Although tolerance mechanisms are necessary for fetal survival, suppressed cellular immunity renders pregnant women susceptible to severe infection with certain pathogens (3–7). Augmented innate immunity may partially offset the reduced adaptive immune protection in pregnancy (8, 9). Following delivery, however, the maternal immune system is released from the constraints of pregnancy.

The postpartum period is associated with alterations in the severity of certain autoimmune diseases and enhanced control of several chronic viruses. For example, multiple sclerosis symptom severity is often alleviated during pregnancy and exacerbated following delivery (10). Similarly, women persistently infected with hepatitis C virus (HCV) or hepatitis B virus (HBV) may experience a precipitous decrease in viral load in the months following delivery (11–13). Factors that contribute to the enhanced postpartum viral control are not fully understood, although in the context of HCV infection, the decrease in viral load was linked in two women to the emergence of viral variants with escape mutations in class I epitopes, suggesting a restoration of virus-specific CD8$^+$ T-cell selection pressure (11).

There is evidence, however, that a brief lag phase in T-cell function may occur following delivery. Although absolute numbers of T cells normalize by 1-mo postpartum (14, 15), these T cells may possess functional effector deficits such as decreased IFN-γ production (15). We hypothesized that maternal innate immune activation extends beyond gestation, thus compensating for the lag phase in postpartum T-cell immunity. We tested this hypothesis by comparing the transcriptional profiles of total peripheral blood mononuclear cells (PBMCs) from healthy mothers following delivery in relation to nonpregnant control subjects (NPCs). We identified an IFN-stimulated gene (ISG) signature that was primarily expressed in CD14$^+$ cells and depended on the interferon-λ3 (*IFNL3*, also known as IL28B) single-nucleotide polymorphism (SNP) rs12979860. Together these data suggest that *IFNL3* genotype may influence innate immunity following delivery.

Results

We used a systems biology approach to identify gene expression patterns and pathways that may contribute to postpartum control of viral infections. PBMCs were collected 2 wk following delivery from two healthy mothers, UM07 and UM08, and two NPCs, NPC354 and NPC357. All NPC subjects were women in their childbearing years who had never been pregnant. Postpartum genes enriched at least twofold over NPCs were included in the analysis; 982 genes were differentially expressed in both UM07 and UM08. Further analysis of these genes by DAVID bioinformatics indicated that the top five enriched biological processes were related to immune response [Gene Ontology (GO):0006955], positive regulation of defense response to virus by host (GO:0002230), response to virus (GO:0009615), defense response to virus by host (GO:0050691), and regulation of immune effector process (GO:0002697) (Fig. 1*A*). Remarkably, all five

Significance

In this study, we examined the possibility that the maternal innate immune system is modulated following delivery. We identified an interferon-stimulated gene signature that was primarily expressed in CD14$^+$ cells circulating in the peripheral blood. Postpartum antiviral gene expression depended on the interferon-λ3 (*IFNL3*) single-nucleotide polymorphism rs12979860, which suggests that *IFNL3* genotype may influence a mother's innate immune response following delivery.

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[1]To whom correspondence may be addressed. Email: jonathan.honegger@nationwidechildrens.org or arash.grakoui@emory.edu.

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biological processes were associated with immunity, and three of these were specifically related to antiviral immunity.

Due to the number of antiviral genes within the "defense response to virus" biological process, this category was investigated further. The enriched antiviral defense genes encoded proteins with antiviral effector function (IFIT1, IFIT2, IFIT3, and OAS1) and signaling function (STAT1, STAT2, MAVS, and TLR7). Interestingly, the IFN-α/β signaling pathway was enriched postpartum. Network analysis identified STAT1 and STAT2 to be the regulatory nodes within the network (Fig. 1*B*). STAT1 and STAT2 are key modulators of type I and type III IFN signaling, and together these data are consistent with up-regulated antiviral IFN signaling in peripheral blood cells following delivery in UM07 and UM08.

We then examined the duration of antiviral gene enrichment following delivery. Gene expression analysis was performed on PBMCs from UM07 and UM08 obtained 2-, 4-, 12-, and 24-wk following delivery and compared with the gene signatures from NPCs (Fig. 1*C*). Interestingly, many antiviral genes were enriched as late as 24 wk following delivery. Expression patterns varied from transient expression 2- to 4-wk postpartum, as in the case of *MAVS* and *STAT2*, to prolonged expression from 12 to 24 wk following delivery, as exemplified by *IFIT1*, *IFIT2*, and *IFIT3* (Fig. 1*C*).

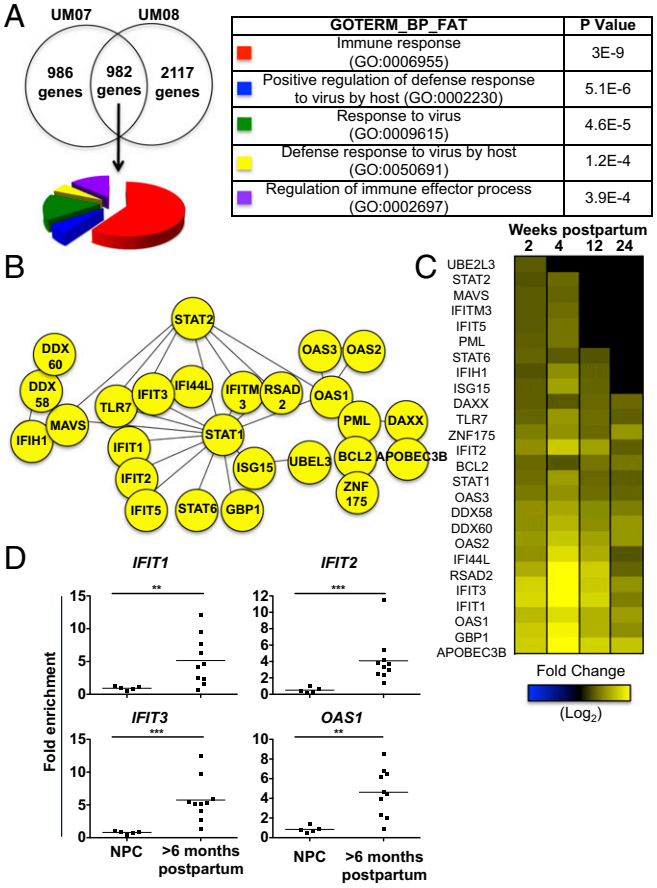

Fig. 1. Antiviral defense genes are sustained greater than 6-mo postpartum in PBMCs from healthy mothers. (*A*) Top five biological processes increased 2-wk postpartum over NPCs. Fold-changes were calculated relative to the average of NPC subjects. DAVID Bioinformatics Resource was used for functional annotation, and gene accession numbers for biological processes are listed. (*B*) Network analysis of antiviral genes enriched 2-wk postpartum. Each node represents one gene within the biological process. (*C*) Heat map of antiviral gene expression in total PBMCs obtained from UM07 and UM08 between 2- and 24-wk postpartum. Fold-changes were calculated relative to the average transcript levels from NPC354 and NPC357. (*D*) RT-PCR analysis of antiviral genes. Gene expression levels from bulk PBMCs are normalized to the *GAPDH* housekeeping gene and calculated as fold-change relative to healthy NPC354 by using the formula $2^{-\Delta\Delta Ct}$. Each dot represents one individual donor. (Mann–Whitney test, $^{**}P < 0.01$, $^{***}P < 0.001$). Time points at which postpartum samples were collected are indicated in Table S1.

Fig. 1*D*). Because the transcriptome analysis indicated that *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* remained elevated 6-mo postpartum in PBMCs from UM07 and UM08, we extended these findings in additional healthy mothers. PBMCs were collected from 10 mothers at late time points following delivery (Table S1). RT-PCR was used to compare transcript expression of ISGs. *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* remained enriched beyond 6-mo postpartum relative to PBMCs from NPCs (Fig. 1*D*), which was in alignment with the postpartum ISG enrichment observed in UM07 and UM08. PBMCs were also isolated approximately 5 y following delivery from two mothers, UM1001 and UM1002. Surprisingly, 5-y postpartum all analyzed ISGs were enriched relative to NPC samples.

We next set out to determine whether postpartum antiviral gene expression was enriched in particular subsets of the circulating mononuclear cells. The 2-wk postpartum time point was chosen because of the relative fold increase in ISGs observed in the microarray analysis. We first confirmed that ISG levels were induced in early postpartum PBMCs from three mothers by RT-PCR. *IFIT1* levels were similar between UM07 and UM08, with both mothers displaying approximate sixfold increases relative to NPCs. *IFIT2* levels were enriched by nearly sixfold in UM07 but were not significantly enriched in UM08, whereas *IFIT3* levels were enriched by approximately fourfold in UM08 but not UM07 (Fig. 2*A*). Similarly, levels of *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* were enriched in total PBMCs obtained from UM332 following delivery (Fig. 2*A*).

$CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, $CD19^+$ B lymphocytes, and $CD14^+$ monocytes were separated based on surface marker expression and analyzed for antiviral gene expression. Surprisingly, the highest postpartum transcript levels of genes encoding IFIT1, IFIT2, IFIT3, and OAS1 were detected within the isolated $CD14^+$ population (Fig. 2*A*). Transcript levels in $CD14^+$ cells between NPCs did not vary by more than approximately twofold, whereas *IFIT1, IFIT2, IFIT3*, and *OAS1* levels were elevated postpartum in UM07, UM08, and UM332. In contrast, transcript levels of these genes in cells expressing CD4, CD8, CD19, or in the remaining cell subsets did not appear to exceed the range of expression seen in NPCs (Fig. 2*A*). To ensure that the postpartum ISG enrichment observed in total PBMCs was not solely due to elevated numbers of $CD14^+$ cells, flow cytometry was used for analysis of cell frequency. The frequencies of $CD14^+$ cells within total PBMCs used for transcript analysis were not significantly different between NPCs and postpartum subjects (Fig. 2*B*).

To extend the findings from these three mothers, we analyzed antiviral gene expression in $CD14^+$ cells from an additional 14 mothers and 10 NPC subjects. Characteristics of the mothers and NPCs enrolled in this study are shown in Tables S1 and S2. $CD14^+$ cells were isolated, and the levels of *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* were examined by RT-PCR. NPC transcript levels were tightly clustered for each gene tested (Fig. 3*A*), whereas highly variable antiviral transcript levels were observed postpartum. Multiple factors could influence postpartum gene expression, so we examined a potential role for genetic factors.

The *IFNL3* SNP rs12979860 is strongly associated with the level of ISGs found within HCV-infected livers. TT or CT genotypes at

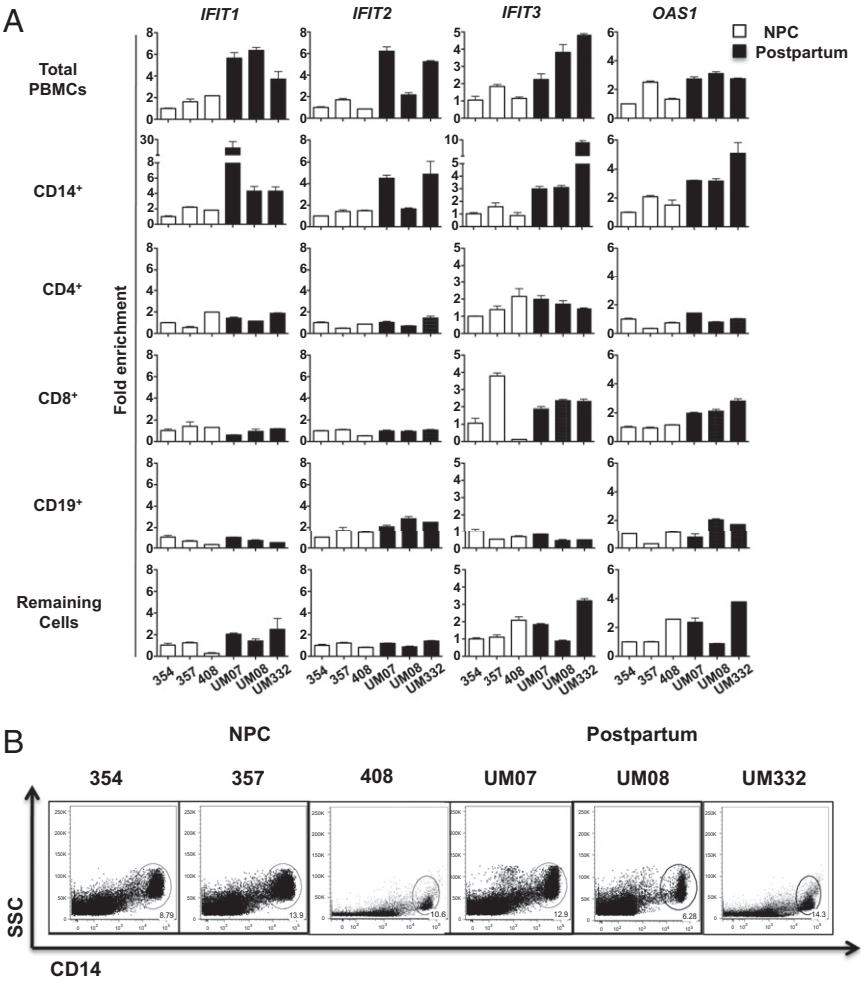

Fig. 2. Antiviral defense genes are enriched postpartum in CD14$^+$ cells. (*A*) Antiviral gene expression levels of *IFIT1, IFIT2, IFIT3*, and *OAS1* from isolated CD14$^+$, CD4$^+$, CD8$^+$, and CD19$^+$ cell populations were analyzed by RT-PCR and shown as fold-change relative to NPC354 (bars represent SEM). (*B*) Frequency of CD14$^+$ cells in total PBMCs used for transcript analysis in *A*.

this SNP are linked to high ISG levels, whereas patients with a CC genotype generally express low levels of ISGs (18). Given the relationship between this SNP and antiviral gene expression, we hypothesized that the antiviral transcript levels from postpartum CD14$^+$ cells could also be stratified based on *IFNL3* genotype at rs12979860. Donor *IFNL3* genotype was determined by using a PCR-based allelic discrimination assay (Fig. 3*A*). Donors with TT or CT genotypes were grouped together because of their similar patterns of antiviral gene expression. Purified CD14$^+$ cells from NPCs displayed consistent baseline levels of ISG transcripts, regardless of *IFNL3* genotype (Fig. 3*A*). Interestingly, the transcript levels of *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* within isolated CD14$^+$ cells from mothers homozygous for the CC alleles were not significantly different from genotype CC or non-CC NPCs (Fig. 3*A*).

In comparison, CD14$^+$ cells isolated from women with either TT or CT genotypes at rs12979860 displayed significantly enriched transcript levels of *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1*, both relative to NPC and homozygous CC genotype postpartum samples. It is interesting to note that within the postpartum TT/ CT cohort, the relative enrichment of the highest data point within each analyzed gene was not recurrently obtained from the same donor. The donor displaying the highest postpartum *IFIT1*, *IFIT2*, *IFIT3*, and *OAS1* transcript level was UM07, UM1001, UM332, and UM06, respectively (Fig. 3*A*). These results indicate that postpartum antiviral transcript levels in CD14$^+$ cells from uninfected mothers may be stratified according to *IFNL3* genotype at SNP rs12979860.

Since the initial microarray was performed on PBMCs isolated following delivery from two mothers with CT alleles at SNP rs12979860 (Fig. 1 *A*–*C*), we next investigated whether ISG enrichment in postpartum PBMCs from homozygous CC individuals would more closely resemble NPCs. Total PBMCs were isolated 2-wk postpartum from two CC donors, UM10 and UM12, and compared with the same two NPC donors (NPC354 and NPC357) that were used for the gene expression analysis in Fig. 1. Analysis of antiviral genes indicated little to no enrichment from postpartum CC donors (Fig. 3*B*), a finding in agreement with our analysis of purified CD14$^+$ cells (Fig. 3*A*). This lack of enrichment in CC donors was in stark contrast to the high level of antiviral transcripts observed 2-wk postpartum in CT donors UM07 and UM08 (Fig. 3*B*).

We next stratified the level of PBMC ISG enrichment observed between 6-mo and 5-y postpartum (Fig. 1*D*) based on *IFNL3* genotype. There was a trend toward higher ISG expression in mothers with CT or TT genotypes vs. a CC genotype at late postpartum time points (Fig. S1). However, it is important to note that only three samples were available from mothers with a CC genotype. Taken together, PBMC ISG expression is prolonged at least 6-mo postpartum in mothers with a CT or TT genotype (Fig. 1 *C* and *D*), but resembles baseline NPC levels 2-wk postpartum in mothers with a CC genotype (Fig. 3*B*).

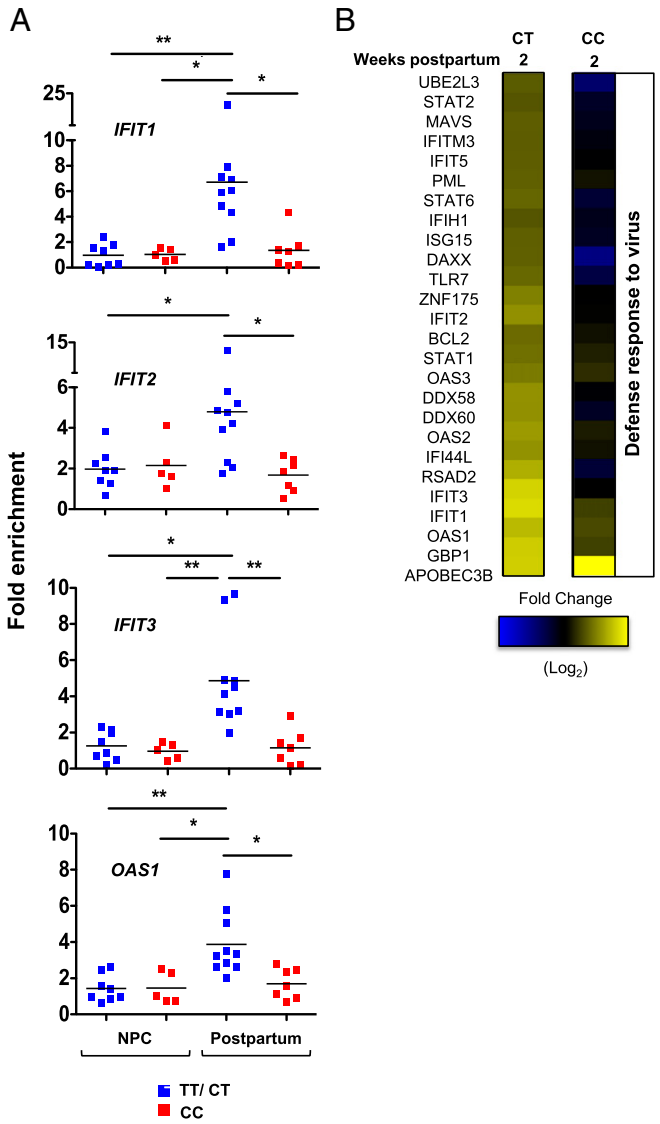

Fig. 3. Postpartum antiviral defense transcript enrichment can be stratified based on allele expression at *IFNL3* SNP rs12979860. (*A*) RT-PCR analysis of antiviral genes. Gene-expression levels from isolated CD14$^+$ cells are expressed as fold-change relative to healthy NPC354. Each dot represents one individual donor. (Kruskal–Wallis test with subsequent Dunn's test for pairwise comparison, $*P < 0.05$, $**P < 0.01$). (*B*) Gene-expression analysis from total PBMCs obtained postpartum from two mothers with CT genotypes (UM07 and UM08) and two mothers with CC genotypes (UM10 and UM12). Fold-changes were calculated relative to the average transcript levels from NPC354 and NPC357.

Discussion

We initially hypothesized that innate antiviral immunity may be augmented in the weeks after childbirth to protect women from infections while adaptive immune function recovers. Here, we found that some women exhibited up-regulation of IFN-stimulated antiviral genes in CD14$^+$ cells after delivery. Strikingly, this signature was limited to women with a particular *IFNL3* rs12979860 genotype. These findings raise important questions about the mechanisms and potential benefits or harms of protracted IFN signaling in CD14$^+$ cells after childbirth.

Interestingly, the observed effects of this SNP on ISG expression in circulating CD14$^+$ cells after delivery (Figs. 1*C* and 3*A*) parallel findings in HCV-infected livers (18). The *IFNL3* SNP at position rs12979860 was originally identified by genomewide association studies to be predictive of spontaneous and IFN treatment-induced resolution of infection with HCV (19–22). HCV-infected individuals with the favorable homozygous CC genotype exhibit low intrahepatic ISG levels, whereas those with the unfavorable CT or TT genotypes exhibit higher intrahepatic antiviral gene expression (18–22). Although the association between impaired clearance of HCV and high intrahepatic ISG levels is counterintuitive, it has been suggested that IFN exerts its protective effect early after infection, whereas prolonged IFN signaling may lead to "interferon exhaustion." Indeed, in a mouse model of chronic infection, blockade of type I IFN before infection with lymphocytic choriomeningitis virus clone 13 enhanced viral load during the acute phase of infection, but led to viral clearance at later time points (23, 24).

Some evidence suggests that protracted IFN signaling may also impair CD14$^+$ function. Tilton et al. described an association between ISG enrichment in CD14$^+$ cells and decreased proinflammatory cytokine production in patients infected with HIV (25). This study hints that in certain settings, the postpartum antiviral transcript enrichment in CD14$^+$ cells could actually be less favorable because of decreased cytokine production. However, we cannot rule out the possibility that enrichment of antiviral genes after pregnancy associated with the *IFNL3* CT or TT genotype could be advantageous for certain infections.

It is tempting to speculate that the *IFNL3* genotype effects on IFN signaling could influence other postpartum complications including bacterial infections, autoimmune diseases, or even depression. Puerperal sepsis, a bacterial infection originating in the uterus following delivery, is the leading infectious cause of maternal death worldwide (26). Type I IFN signaling has been associated with increased severity of infection with a number of relevant pathogens, including staphylococcal and *Pseudomonas* species (27, 28). Likewise, elevated levels of type I IFN are associated with pathogenesis of certain autoimmune diseases such as systemic lupus erythematosus (SLE) (29). Because SLE flares are common during and following pregnancy (30), perhaps the basal levels of IFN signaling in mothers with a CC genotype could influence flare severity. In contrast, patients with multiple sclerosis are often treated with recombinant type I IFN (29). Some women are prone to postpartum relapses (10), suggesting that prolonged IFN signaling could be protective following delivery. Further, depression is a side effect of therapeutic treatment with exogenous IFN-α (31) and has been associated with aberrant proinflammatory cytokine production (32). A CC genotype could potentially be favorable for women prone to postpartum depression. Although we have not studied these potential connections, they are intriguing possibilities worth examining in the future. It will be exciting to learn which infections and diseases are impacted by *IFNL3* genotype and the variable CD14$^+$ ISG expression following delivery.

The mechanisms underlying the prolonged and selective ISG up-regulation in CD14$^+$ cells of *IFNL3* CT/TT women after childbirth remain elusive. First, the mechanism by which the *IFNL3* rs12979860 SNP exerts an effect on ISG signaling in any cell type is unsettled, with uncertainty about whether the SNP is actually functional or whether its effect is mediated by other *IFNL* SNPs in high linkage disequilibrium (33). Individuals with a ΔG genotype (vs. T) at the *IFNL4* variant rs368234815 express a transcript encoding the IFN-λ4 protein, which has been demonstrated to induce IFN signaling (33, 34). All of the NPC subjects and mothers from our study were genotyped at rs368234815, and in each individual there was a direct correlation between *IFNL3* SNP rs12979860 and *IFNL4* rs368234815 genotypes (Table S1). Second, an effect of *IFNL3* genotype on ISG expression has not been previously described for CD14$^+$ cells to our knowledge. Monocytes are known to increase in both frequency and activation marker expression during pregnancy and labor (9, 15), with roles in placental vascularization, initiation of labor, and potentially immune protection during a period of reduced adaptive immunity. Nonetheless, the stimulus for continued IFN signaling in CD14$^+$ cells after delivery is unknown.

In human pregnancy, fetal-derived tissues come in direct contact with maternal blood, which is known as hemochorial placentation. Placental syncytiotrophoblasts, a primary cell type of the placenta, invade maternal blood vessels, which allows maternal blood and blood cells to directly contact semiallogeneic syncytiotrophoblasts (35). Because placental cells are capable of IFN production (36) and physical interactions between syncytiotrophoblasts and maternal $CD14^+$ monocytes involving ICAM-1, LFA-1, and fractalkine have been described (37–39), it is possible that stimulation of $CD14^+$ cells in vivo requires cell-to-cell contact. This finding could explain why enriched antiviral transcripts were primarily expressed within the $CD14^+$ population and not globally in all circulating cell types (Fig. 2*A*). In addition, a proinflammatory cytokine milleu is thought to contribute to uterine contraction and delivery. Following delivery, uterine tissue is regenerated during the wound healing process. The placental proinflammatory environment and uterine tissue repair associated with childbirth could conceivably provide a transient stimulus for ISG expression, but it is difficult to envision how these processes would support enhanced ISG expression for years after delivery as seen in mothers with an *IFNL3* CT/TT genotype (Fig. 1*D* and Fig. S1).

The duration of ISG expression suggests that the enriched postpartum ISGs may depend on continuous stimulation or reflect a permanent change within the $CD14^+$ cell subset. It is known that microchimeric fetal cells take residence in the mother during pregnancy and may persist in the mother for years following delivery (40). Microchimeric cells could be targets for maternal allogeneic T cells, potentially resulting in continuous low-grade inflammation and ISG expression.

An alternate hypothesis stems from the observation that epigenetic modification is not uncommon during pregnancy. For example, a cluster of 46 microRNAs, called the chromosome 19 miRNA cluster (C19MC), is induced by epigenetic modification within the placenta (41, 42). An interesting observation is that many genes involved in innate immunity and reproduction are located along chromosome 19, which also contains the *IFNL3* SNP rs12979860. Two genes within this region, *Akt2* and *IRF3*, specifically modulate ISGs (43, 44). If this region were epigenetically modified following delivery, a transcriptionally accessible conformation could be maintained in $CD14^+$ cells of women with CT or TT genotypes, but remain inaccessible to transcriptional machinery in mothers with a CC genotype. Continuous expression of *Akt2* and *IRF3* could potentially contribute to the prolonged ISG expression observed following delivery. Further work will be required to determine the stimulus for prolonged ISG expression in $CD14^+$ cells and the factors that contribute to differences in ISG expression from TT/CT and CC genotypes after delivery.

Overall, we identified a prolonged ISG signature that was primarily expressed in $CD14^+$ cells following delivery in healthy women. Postpartum ISG enrichment depended on *IFNL3* genotype. Together these data may have important ramifications for our understanding of the role of polymorphism-dependent IFN signaling, and the maternal response to infections and other inflammatory diseases following delivery.

Methods

Study Approval. Healthy nonpregnant control subjects and mothers were recruited from the Yerkes National Primate Research Center Healthy Donor Protocol under the approval of the institutional review board at Emory University. Donors provided written consent for blood donation. Healthy mothers (negative for hepatitis B surface antigen, hepatitis C antibody, and HIV antibody) were also recruited from the Ohio State University Substance Treatment, Education and Prevention in Pregnancy Program. Following informed consent, blood collections were performed at Nationwide Children's Hospital in Ohio under the approval of the institutional review boards at Ohio State University and Nationwide Children's Hospital. Donor characteristics are highlighted in Tables S1 and S2.

Blood Collection and Processing. Blood samples were collected in Vacutainer tubes (BD), and PBMCs were isolated by using Ficoll gradient centrifugation. Cells were suspended at a concentration of 5×10^6 cells per mL in FBS (HyClone) containing 10% (vol/vol) dimethyl sulfoxide (DMSO) and stored at −150 °C.

RNA Purification for Microarray Analysis. PBMCs were stored in 350 μL of RLT buffer (Qiagen) containing 1% β-mercaptoethanol. Total RNA extraction was performed by using the RNeasy mini kit according to the manufacturer's specifications; on-column DNase digestion was also performed to remove genomic DNA. RNA integrity of the extracted RNA was assessed by Agilent Bioanalyzer (Agilent Technologies) capillary electrophoresis on an RNA 6000 NanoChip; all samples had an RNA Integrity Number score of 8.5 or higher. Quantitative analysis was performed by using the NanoDrop 2000 spectrophotometer (Thermo Scientific).

Microarray Hybridization. For each individual sample, cDNA synthesis and amplification was performed by using the NuGEN Ovation Pico WTA V2 system (NuGEN). Briefly, 25 ng of total RNA were used for cDNA synthesis followed by whole transcriptome amplification by NuGEN's Ribo-SPIA technology. The Ribo-SPIA technology uses DNA/RNA chimeric primers to amplify cDNA isothermally maintaining the stoichiometry of the input RNA. The amplified single-stranded DNA was purified by using the AMpure XP beads (Beckman). Qualitative and quantitative analyses were performed on the Bioanalyzer and NanoDrop, respectively, to assess the size distribution of the amplified DNA and quantity. Five micrograms of the amplified DNA were used for biotinylation and fragmentation by using the NuGEN Ovation Encore Biotin Module (NuGEN).

Microarray Analysis and Bioinformatics. Data were analyzed by using TIBCO Spotfire with OmicsOffice for Microarrays (Integromics Biomarker Discovery). Primary microarray data has been submitted to Gene Expression Omnibus in accordance with proposed Minimum Information About a Microarray Experiment standards. DAVID Bioinformatics Resources and Ingenuity Pathways Analysis was used to identify biological pathways and processes that were enriched in postpartum samples over nonpregnant controls. These programs analyze and categorize gene sets within known biological pathways or networks.

Cell Purification. $CD14^+$ cells were isolated by using human CD14 MicroBeads and MACS columns according to the manufacturer's instructions (Miltenyi Biotech). Other subpopulations were purified by using CD8, CD4, and CD19 Pan B-cell Dynabeads for human cell isolation, also according to the manufacturer's instructions (Life Technologies).

RT-PCR. Total RNA was extracted by using an RNeasy Mini Kit and treated with an RNase Free DNase Set (Qiagen) according to the manufacturer's instructions. Reverse transcription reactions were performed by using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Gene expression levels were determined by using the Power SYBR Green PCR Master Mix (Applied Biosystems) on an Applied Biosystems 7500 apparatus.

Allelic Discrimination Assays. Genotypes of NPC and postpartum subjects at *IFNL3* SNP rs12979860 and *IFNL4* were determined as described (18, 34). Briefly, genomic DNA was isolated from patient samples by using the PureLink Genomic DNA Mini Kit (Qiagen) per the manufacturer's protocol. Allelic discrimination was carried out by using Taqman Genotyping Master Mix. Samples were run on an ABI 7500 Real-Time PCR instrument (Applied Biosystems), and donor genotype was assigned by automated algorithm included in the SDS v1.3.1 software suite (Applied Biosystems).

Flow Cytometric Analysis. PBMCs were stained with antibodies to CD3 (FITC, clone UCHT1; Beckman Coulter), CD8 (PE, clone SK1; BioLegend), HLA-DR (PerCP – Cy5.5, clone L243; BioLegend), CD19 (violet fluor 450, clone HIB19; Tonbo Biosciences), and CD14 (PE-Cy7, clone M5E2; BD Biosciences) for analysis of cell frequencies and isolation purity.

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