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Pro-inflammatory immune cell gene expression during the third trimester of pregnancy is associated with shorter gestational length and lower birthweight

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Abstract

Problem: Altered maternal immune function predicts risk for shorter gestation and low birthweight. Few studies examine associations between prenatal immune cell gene expression and gestational length or birthweight. No studies examine which cell types drive associations. The purpose of this study is to explore associations between peripheral blood immune cell gene expression and gestational length and birthweight, using transcript origin analysis.

Method of study: Eighty-nine women were drawn from the Community Child Health Network cohort. Third trimester maternal dried blood spots were used for genome-wide transcriptional (mRNA) profiling. Gestational length and birthweight were obtained from medical charts. Covariates were age, race/ethnicity, pre-pregnancy body mass index, smoking, gestational age at blood sampling, and pregnancy infections. Associations between gene expression profiles and gestational length and birthweight were tested using general linear models. The Transcription Element Listening System (TELiS) bioinformatics analysis quantified upstream transcription factor activity. Transcript origin analysis identified leukocyte subsets mediating observed effects.

Results: Shorter gestation was predicted by increased NF-kB (TFBM ratio = −0.582 ± 0.172, $P < .001$) and monocyte activity (diagnosticity score = 0.172 ± 0.054, $P < .001$). Longer gestation was associated with increased dendritic cell activity (diagnosticity score = 0.194 ± 0.039, $P < .001$). Increased AP-1 activity predicted lower birthweight (TFBM ratio = −0.240 ± 0.111, $P = .031$). Dendritic cells and CD4+ and CD8+ T cells predicted birthweight-related gene expression differences (diagnosticity score P's < 0.021).

Conclusion: Higher third trimester pro-inflammatory gene expression predicted shorter gestation and lower birthweight. Variations in monocyte and dendritic cell biology contributed to both effects, and T-cell biology contributed to higher birthweight. These analyses clarify the role of myeloid/lymphoid lineage immune regulation in pregnancy outcomes.

KEYWORDS
birthweight, gene expression, gestational length, immune cells, mRNA

1 | INTRODUCTION

Inflammatory and immune processes play key regulatory roles across pregnancy,[1,2] and evidence from human and animal studies suggests that dysregulation of inflammatory and immune processes during pregnancy is associated with risk for adverse pregnancy outcomes, such as shorter gestation or low birthweight.[2-4] Infections during pregnancy and lifestyle factors associated with increased inflammation, for example obesity and smoking, have been associated with risk for preterm birth.[2,3,5-11] And elevated levels of peripheral inflammatory markers have been observed in women who went on to have preterm labor compared with those who did not.[7,12-14] There is also evidence that inflammatory pathways could play a role in risk for low birthweight. For example, preeclampsia, an inflammation-associated hypertensive disease of pregnancy,[15-17] increases risk for small for gestational age infants. And some studies have reported associations between elevated pro-inflammatory markers across pregnancy and risk for lower birthweight.[18-23]

Systemic changes in immune activity are regulated in large part by upstream changes in immune cell gene expression. To date, the majority of immune-related gene expression studies have focused on expression in the placenta, fetal membranes, or associated tissues,[24-27] and have generally observed increased pro-inflammatory gene expression predicting risk for shorter gestational length and lower birthweight. A few studies have detected differences in immune cell gene expression between women who did or did not deliver preterm.[28-31] In a sample of 1878 pregnant women, gene set enrichment analysis, which analyzes mRNA patterns based on prespecified gene sets, that is groups of genes that share common biological function, regulation, or chromosomal locations,[32] was used to show that compared with women who delivered term, those who delivered preterm had altered third trimester inflammatory gene sets and pathways, independent of birthweight and early pregnancy urinary tract infections. This included up-regulated leukocyte migration, NF-kB, cytokines and cytokine receptors, and toll-like and NOD-like receptor signaling, and down-regulated genes associated with T-cell activation. Another analysis of data obtained from three separate studies (combined N = 339), each of which collected maternal blood immediately before or during delivery, reported that, compared with women who delivered term, women who delivered preterm had immune cell gene expression patterns indicative of up-regulated innate immunity pathways and down-regulated adaptive immunity pathways.[31] Collectively, these results suggest that up-regulated innate immunity and inflammatory gene expression during later pregnancy are associated with shorter gestation. However, studies that compare immune cell gene expression between women with and without preterm delivery have a number of limitations. For example, there is evidence that immune cell gene expression profiles vary as a function of gestational age,[33] and appropriately matching women who deliver preterm with those who do not on gestational age at sampling can be a challenge,[29] making it difficult to determine whether differences are due to preterm delivery or sample collection timing. Additionally, most studies cannot account for medical factors associated with preterm delivery that also affect immune cell gene expression, such as glucocorticoid treatment[29] or presence of infection.[31] These confounding factors are somewhat mitigated if gestational length is examined as a continuous variable, rather than comparing term and preterm pregnancies. However, no studies were identified that examined associations between immune cell gene expression patterns and gestational length. In addition, no studies have examined associations between prenatal inflammatory gene expression in blood and birthweight.

In addition, previous work on peripheral immune cell gene expression during pregnancy focused on general differences in gene expression in whole blood samples[28] (For an exception, see Ref. 29). This approach, however, does not take into consideration the many kinds of immune cells that play roles in maintaining pregnancy and/or initiating processes that lead to labor and delivery. Natural killer cells, monocytes, macrophages, and dendritic cells, various classes of T cells, and neutrophils have all been identified as playing important roles during pregnancy.[34-40] Without understanding which immune cells in the maternal circulating blood cell pool contribute to the aggregate transcriptomic association with gestational length or low birthweight, it is difficult to determine which mechanistic pathways are involved or identify targets for clinical intervention. One way to approach this question is to apply a specialized bioinformatic method called transcript origin analysis, which was developed to identify the specific cell subsets that contribute to differential gene expression profiles measured within an aggregate cell population.[41]

As such, the purpose of this study was to explore links between pro-inflammatory gene expression and cellular activation profiles in maternal blood samples collected during the third trimester of pregnancy and gestation length and birthweight. A diverse, low-income sample of women was recruited as part of the Community Child Health Network (CCHN), a prospective study of chronic stress and postpartum outcomes. These analyses focus on the subset of women who became pregnant again over the follow-up, and for which banked third trimester dried blood spot (DBS) samples were available for immune cell gene expression assay. We hypothesized that up-regulated pro-inflammatory gene expression would be associated with shorter gestational length (independent of birthweight) and lower birthweight (independent of gestational length), as tested using bioinformatic measures of transcription factor activity derived from genome-wide transcriptional profiling data.[42]

2 | METHODS

2.1 | Participants

Data were drawn from the Community Child Health Network (CCHN), funded by the Eunice Kennedy Shriver National Institutes of Child Health and Human Development (NICHD) and National Institutes of Health (NIH). The larger CCHN cohort consists of 2510 low-income Black, White, and Latina women recruited from five sites across the USA (Washington, DC; Baltimore, MD; Los Angeles, CA; Lake County, IL; eastern North Carolina). Women were

recruited following the birth of a child, as per eligibility criteria and recruitment procedures reported elsewhere (Figure 1).[43,44] First assessments occurred at 1 month postpartum and continued every 6 months up to 2 years after the birth of the index child (n = 2089). At some point over the follow-up, 416 participants (20%) indicated that they had become pregnant again. Of those, 343 (82%) consented to participate in the subsequent pregnancy follow-up study, and 250 (73%) completed an assessment during the third trimester of pregnancy. Of the 250 participants, 234 (94%) consented to providing a dried blood spot (DBS) sample, which were used in the original study to assay blood metabolic indicators (data not presented). A total of 89 women (38%) had banked DBS available for additional assays, and had information available on birth outcomes, that is gestational length or birthweight. As such, our final sample consisted of 89 women who became pregnant over the follow-up and had both banked DBS and birth outcome data. Participants provided informed, signed consent to participate, and all protocols followed Declaration of Helsinki procedures. Study procedures and protocols were reviewed and approved by the Institutional Review Boards of all community and academic institutions associated with CCHN.[43]

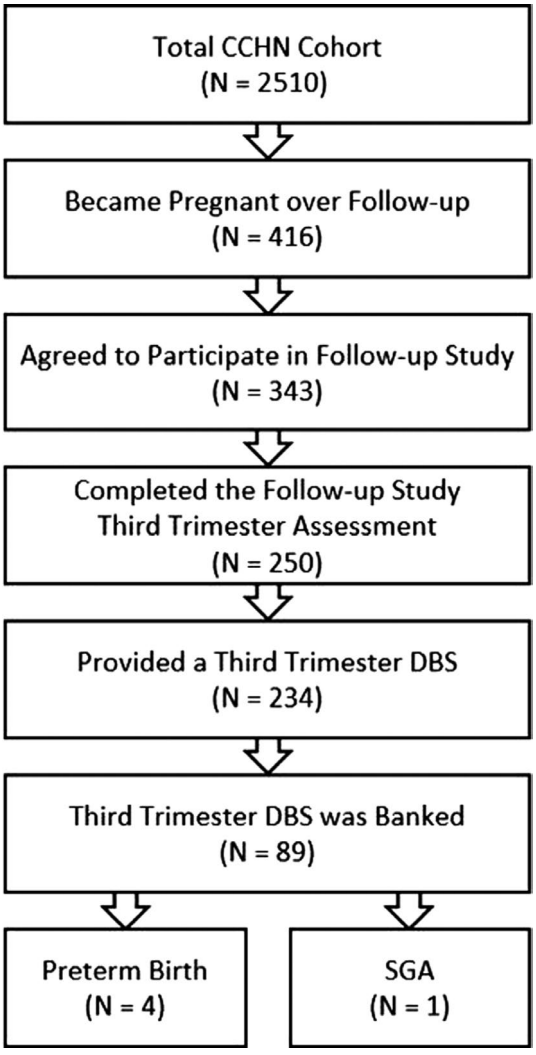

FIGURE 1 Participant recruitment and retention flowchart

2.2 | Procedure

Women completed structured interviews in their homes before the subsequent pregnancy (preconception) which was at 1, 6, and 12 months after the birth of a child, and during the second and third trimester of the subsequent pregnancy. Interviews were conducted in English or Spanish, with attempts to match interviewer and participant ethnicity. Dried blood spots (DBS) were collected during the third trimester of pregnancy, and assayed for inflammatory gene expression (mRNA). Both gestation length (weeks) and birthweight (g) were obtained from medical chart review following delivery.

2.3 | Inflammatory gene expression

A non-fasting blood sample was collected onto a DBS card and assayed for genome-wide transcriptional profiles using RNAseq. Briefly, a finger was lanced and capillary blood collected on filter paper. DBS cards were allowed to dry for 30 minutes, and then stored at −30°C until a subset of each sample was assayed for metabolic markers (data not reported here). Remaining DBS were stored at −80°C and shipped to the UCLA Social Genomics Core Laboratory for RNA extraction as previously described.[45] RNA samples were converted to cDNA libraries using the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen Inc) and sequenced using an Illumina HiSeq4000 instrument (Illumina Inc) in the UCLA Neuroscience Genomics Core Laboratory, following the manufacturers' standard protocol. All samples passed endpoint quality assurance thresholds for DBS RNAseq samples, including >10 million single-strand 65-nucleotide reads per sample, >90% of reads aligning to the reference human genome, and a correlogram average profile $r \geq .80$. Gene expression values were normalized to transcript counts per million total transcripts and $\log_2$-transformed for analysis by linear statistical models as outlined below.

2.4 | Covariates

Covariates considered for inclusion in analyses were maternal demographics (age [years] in the third trimester of pregnancy, and maternal race/ethnicity [Black vs all, Latina vs all]), alcohol consumption, smoking, infection reported or treated over the pregnancy (yes/no), gestational age at the third trimester assessment, and pre-pregnancy body mass index (BMI). Pre-pregnancy BMI was included as a covariate because adipose tissue produces inflammatory markers, and pre-pregnancy BMI is associated with inflammatory activity during pregnancy.[46] Maternal obesity is also associated with risk for shorter gestational length and low birthweight.[47] Height and weight measurements were taken at 1, 6, and 12 months postpartum and were used to calculate BMI (kg/m^2), by dividing weight by height-squared. Pre-pregnancy BMI was defined as the BMI value obtained at the last preconception assessment.

Infections are characterized by an immune response and thus altered immune cell gene expression patterns, and infections during pregnancy are associated with risk for pregnancy complications,

such as shorter gestation.[2] Infections reported and treated over the subsequent pregnancy were obtained from medical risks and conditions reported in response to structured questions administered during the second and third trimester interviews. They included whether a care provider had informed the participant they had, for example, a bladder infection, or were "given any medications to treat infections, such as vaginal, bladder, or kidney infections, during this pregnancy?" If women responded "yes" to any of these questions they were coded as having an infection in pregnancy.

Alcohol consumption and smoking are health behaviors associated with altered immune and inflammatory activity,[48,49] and risk for pregnancy complications.[47] Few women reported any alcohol consumption (N = 1, 1%) or any cigarette use (N = 5, 4%) at any point over the subsequent pregnancy. As such, these variables were not included in the analyses, although women who reported alcohol consumption and cigarette use were retained in analyses.

Evidence suggests that immune and inflammatory activity changes systematically over the course of pregnancy,[50,51] and so immune cell gene expression profiles could also be affected by gestational age at blood sampling. Gestational age at blood sampling was calculated based on reported estimated date of conception (based on last menstrual period).

Consequently, final covariates were maternal age, race/ethnicity, pre-pregnancy BMI, infection reported over pregnancy, and gestational age at the time of blood sampling, and gestational length (in models predicting birthweight) or birthweight (in models predicting gestational length).

2.5 | Statistical analyses

Gene expression data were analyzed using standard methods for targeted hypothesis testing in genome-wide transcriptional profiles.[41,42,52-54] Gene expression data were normalized "gene transcripts per million total transcripts" (TPM), floored at 1 TPM, $\log_2$-transformed, and screened to exclude transcripts that varied by <0.5 $\log_2$ units across participants (to remove genes that were generally undetectable or showed no appreciable variation in expression levels across samples). Remaining data were analyzed using a standard general linear statistical model[55] treating $\log_2$ TPM values as the dependent measure, gestational age and birthweight as the key independent measures, and age (in years), BMI (kg/m^2), and race/ethnicity as covariates. Where indicated, ancillary analyses additionally controlled for variations in leukocyte subset prevalence by including as covariates the abundance of mRNAs encoding CD3, CD4, CD8, CD19, CD14, CD16, and CD56. Models were estimated using the Java JAMA matrix algebra package (1.0.3) to implement standard open source numerical algorithms for ordinary least squares parameter estimation.[56] The goal of these analyses was to provide maximum-likelihood point estimates of differential gene expression to serve as input into higher-order bioinformatics analyses that test for transcriptome-wide alterations in the activity of a priori-specified transcription factors and inflammation-related cell types (described below). As such, no statistical significance testing was conducted at the level of individual genes (only at the level of the downstream bioinformatic inferences derived from gene sets defined by point estimates of differential expression magnitude). Point estimates of differential expression were used because they have been found to provide more replicable gene lists than those derived from gene-specific statistical testing.[52,57,58]

Primary analyses used empirical gestational length- or birthweight-associated differences in genome-wide transcriptional profiles to test for differential activity of pro-inflammatory transcription factors, NF-κB and AP-1, as well as the generally anti-inflammatory glucocorticoid receptor (GR). These analyses took as input all genes that showed ≥2.0-fold difference in average expression across the range from 2 SD below the average gestational length (or birthweight) to 2 SD above the average (ie, over the 4-SD range of normal variation). Gene lists were analyzed using a 2-sample variant of the Transcription Element Listening System (TELiS[42]) quantifying the prevalence of NF-κB and AP-1 transcription factor-binding motifs (TFBMs) within the promoters of differentially expressed genes using TFBMs derived from the TRANSFAC database (V$NFKAPPAB_01 and V$AP1_Q6, respectively). Results were averaged across 9 alternative technical specifications involving variations of promoter length (−300, −600, and −1000 to +200 bp) and TFBM detection stringency (MatSim 0.80, 0.90. 0.95). In all analyses, statistical testing was based on standard errors derived from bootstrap resampling of linear model residual vectors (to account for correlation among genes). Two-tailed *P* values <.05 were considered statistically significant.

Secondary analyses used transcript origin analysis[41] to identify the specific leukocyte subsets giving rise to the observed differences in gene expression associated with gestational length- or birthweight. Using reference data from transcriptome profiling of isolated leukocyte subsets (GEO GSE1133), these analyses map the same set of gestational age- and birthweight-associated genes used above to cell type-specific diagnosticity scores that indicate the extent to which those genes are distinctively expressed by CD4+ T cells, CD8+ T cells, B cells, NK cells, monocytes, or BDCA2+ plasmacytoid dendritic cells.[59] Scores are defined as in Cole, Hawkley, Arevalo, Cacioppo[41] computed using the JAMA matrix algebra package, and tested for statistically significant over-representation for each cell type based on standard errors derived from bootstrap resampling of linear model residual vectors (to account for correlation among genes). Two-tailed *P* values <.05 were considered statistically significant.

3 | RESULTS

3.1 | Sample characteristics

Sample characteristics are presented in Table 1. The sample was approximately half Latina, a quarter White, and a quarter Black, and participants were on average 27.9 ± 5.22 years old during the third trimester of pregnancy. Average gestational length was 39.2 ± 3.69 weeks, ranging from 36.3 to 42 weeks gestational age.

Four infants (4%) were born preterm, defined as before 37 weeks gestational age. Average gestational weight was 3413 ± 447 g, ranging from 2105 to 4750 g. Only one infant (1%) fell below the lower end of the normal range for birthweight (2500 g). Gestational age at birth and birthweight was significantly correlated, $r = .303$, $P = .008$, such that infants who were born later also tended to be heavier.

3.2 | Gestational length

Genome-wide analyses of maternal blood samples collected during the third trimester identified 1532 gene transcripts that showed >2.0-fold difference in average expression level over the range of normal variation in gestational length (ie, over the range from 2 SD below the average to 2 SD above average), after controlling for age, race/ethnicity, pre-pregnancy BMI, recent infection status, gestational age at the time of blood sampling, and birthweight (989 transcripts up-regulated in association with gestational length and 543 down-regulated). In analyses of the two key pro-inflammatory transcription factors (NF-kB and AP-1), promoter-based bioinformatic analyses of transcription factor-binding motifs in the promoters of up- vs down-regulated genes linked longer gestational length to lower activity of NF-κB (ie, associating earlier gestation with relatively greater NF-κB activity: $\log_2$ TFBM ratio −0.582 ± standard error 0.172, $P < .001$; Figure 2A). Gestational length showed a paradoxical positive association with the other major pro-inflammatory signaling pathway analyzed, AP-1 (+0.281 ± 0.100, $P = .006$; Figure 2A).

Next, we used transcript origin analysis to identify specific leukocyte subsets that may have contributed to the overall transcriptome differences associated with gestational length. Genes empirically associated with shorter gestational length at birth derived predominately from monocytes (mean cell type diagnosticity score: +0.172 ± 0.054, $P < .001$), whereas genes empirically associated with longer gestational length derived predominately from dendritic cells (+0.194 ± 0.039, $P < .001$; Figure 2B). However, we found no difference in expression of canonical dendritic cell activation markers (*CD83*, *CD40*, *CD80*, *CD86*, *CXCL8*, OX40/*TNFRSF4*) in association with gestational length ($P = .36$ for the 6-gene composite score).

TABLE 1 Sample characteristics (N = 89)

Variable	Mean ± SD or % (N)
Age (y)	27.9 ± 5.22
Race/ethnicity	
Black	27 (24)
Latina	46 (41)
White	27 (24)
Pre-pregnancy BMI (kg/m^2)	29.5 ± 6.07
Infection over pregnancy (yes)	30 (28)
Third trimester gestational age (wk)	32.9 ± 3.69
Gestational length (wk)	39.2 ± 1.05
Birthweight (g)	3413 ± 447

3.3 | Birthweight

Genome-wide analyses identified 1260 gene transcripts that showed >2.0-fold difference in average expression level over the range of normal variation in birthweight (ie, over the range from 2 SD below the average to 2 SD above average) after control for age, race/ethnicity, pre-pregnancy BMI, recent infection status, gestational age at blood sampling, and gestational length (612 transcripts up-regulated and 648 down-regulated). Promoter-based bioinformatics linked greater birthweight to lower activity of AP-1 (ie, associating relative low birthweight with relatively greater AP-1 activity: −0.240 ± 0.111, $P = .031$), but no difference in NF-κB activity (−0.234 ± 0.207, $P = .260$; see Figure 3A).

In analyses of cellular origins of differentially expressed transcripts, genes empirically associated with lower birthweight derived predominately from dendritic cells (+0.157 ± 0.036, $P < .001$) whereas genes empirically associated with higher birthweight derived predominately from CD4+ T cells (+0.081 ± 0.026, $P = .002$), CD8+ T cells (+0.091 ± 0.026, $P < .001$) and dendritic cells (+0.077 ± 0.037, $P = .021$; see Figure 3B). Again, we found no difference in expression of canonical dendritic cell activation markers (*CD83*, *CD40*, *CD80*, *CD86*, *CXCL8*, OX40/*TNFRSF4*) in association with birthweight ($P = .68$ for the 6-gene composite score).

4 | DISCUSSION

The purpose of this study was to extend previous work on associations between maternal immune cell gene expression and birth outcomes by identifying specific pro-inflammatory transcription factors and leukocyte subsets associated with variations in gestational length and birthweight. Using a sample of low-income, diverse women, we replicated previous findings reporting an association between increased pro-inflammatory gene expression during the third trimester of pregnancy and shorter gestational length,[28-31] and also report here a similar association between increased pro-inflammatory immune cell gene expression and lower birthweight, independent of gestational length. However, the specific pro-inflammatory transcription factors implicated in driving the observed transcriptome variations differed by outcome, with elevated NF-κB activity associated with shorter gestational length, and elevated AP-1 activity associated with lower birthweight. Analyses of the specific leukocyte subsets involved implicated dendritic cells in structuring the aggregate blood transcriptome profiles associated with both longer gestational length, independent of birthweight, and greater birthweight, independent of gestational length. CD4+ and CD8+ T-cell subsets were also implicated in greater birthweight, but not longer gestational length. Less favorable birth outcomes were associated with increased expression of monocyte-related genes (gestational length, consistent with other studies[29,31]) and dendritic cell-related genes (birthweight). This is the first study to examine associations between immune cell gene expression profiles and gestational

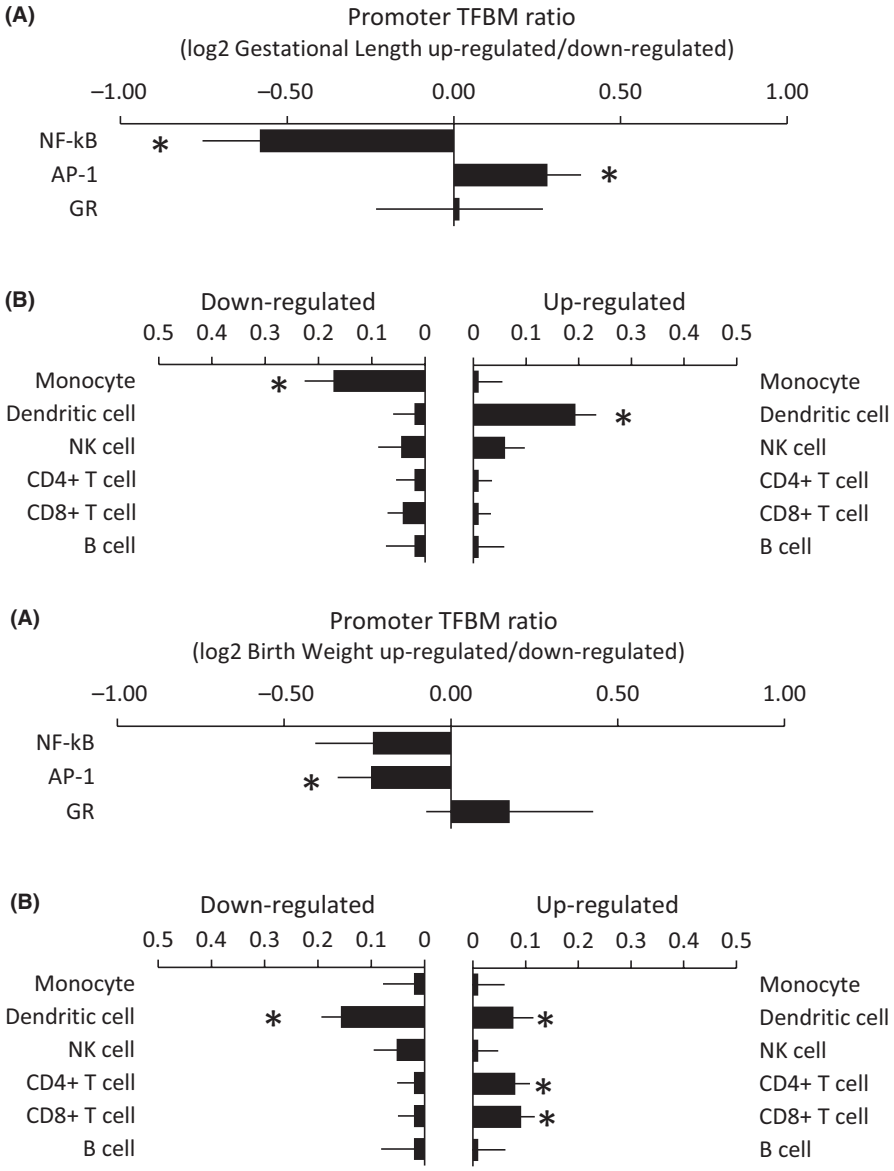

FIGURE 2 A, Promoter-based bioinformatics analyses of transcription factor-binding motifs in promoters of up- vs down-regulated genes associated with gestational length. B, Results of transcript origin analysis to identify leukocyte subsets that could account for overall transcriptome differences associated with gestational length

FIGURE 3 A, Promoter-based bioinformatics analyses of transcription factor-binding motifs in promoters of up- vs down-regulated genes associated with birthweight. B, Results of transcript origin analysis to identify leukocyte subsets that could account for overall transcriptome differences associated with birthweight

length and birthweight in a sample of women who primarily delivered infants of average size at term. Overall, these results are consistent with previous research linking inflammatory processes to premature delivery,[7,12-14] and suggest that these processes also play an important role in the gestational length of healthy, term pregnancies. These results also extend previous findings by identifying specific transcription control pathways and cell types that can be targeted for future mechanistic research. This study also expands the precision of examination of pregnancy outcomes by analyzing the molecular correlates of low birthweight (independent of gestational length), and gestational length (independent if birthweight).

Our findings are consistent with previous research reporting associations between third trimester pro-inflammatory immune cell gene expression and risk for shorter gestational length.[28] Heng et al[28] observed that, during the third trimester of pregnancy, women who went on to have spontaneous preterm birth had up-regulated enriched gene sets that included cytokine signaling, infection and wound healing, leukocyte migration, and NF-kB-regulated pathways. We also observed an association between shorter gestational length and up-regulated pro-inflammatory gene expression via increased activity of the transcription factor NF-kB. Moreover, transcript origin analyses indicated that genes associated with shorter gestational length were primarily derived from monocytes, highlighting the importance of the innate immune system in regulating immune processes related to gestational length.[36,40] These findings and those reported by Heng et al[28] are consistent with the body of work reporting associations with increased peripheral pro-inflammatory immune proteins and shorter gestational length.[7,12-14] Collectively, this suggests that NF-kB and pro-inflammatory gene activation in peripheral blood cells could be implicated in pathways leading to shorter gestational length.

Additional transcript origin analyses indicated that immune cell gene expression associated with *longer* gestational length was primarily attributable to dendritic cells. This is consistent with

research underlying the importance of dendritic cells in maintaining pregnancy. There are shifts in dendritic cell populations and pattern of activation that promote fetal tolerance and maintenance of a Th2 shift during pregnancy.[60-63] This is in part achieved through unique changes in dendritic cell activity toward the end of pregnancy, characterized by increases in dendritic cell activation but without corresponding increased expression of HLA-DR, an MHC II class receptor that recruits and activates T cells.[63] Together, this produces dendritic cells that are active but do not trigger pathways antithetical to immunotolerance, and thus support maintenance of pregnancy. However, in this study we found no significant association between gestational length and expression of a set of canonical dendritic cell activation markers (CD83, CD80, CD86, CD40, OX40, CXCL8/IL-8), either as an aggregate or individually. It is also important to note that the reference cell transcriptomes used in these analyses included only BDCA2+ plasmacytoid dendritic cells and did not explicitly distinguish them from other dendritic cell subtypes (eg, CD1c/BDCA1+ myeloid dendritic cells or CD141/BDCA3+ dendritic cells). As such, the observed difference in dendritic cell transcriptomic activity may reflect changes in cell differentiation or trafficking, rather than classical dendritic cell maturation to an antigen-presenting phenotype. This hypothesis could also account for the paradoxical association observed between increased activity of the pro-inflammatory AP-1 transcription factor and longer gestational length. AP-1 is involved in regulating genes related to dendritic cell migration,[64] and so links between increased AP-1 activity and longer gestational length could indicate increased dendritic cell migration activity into the uterus to help maintain pregnancy.

Heng et al[28] also reported an association between preterm birth risk and third trimester down-regulation of enriched gene expression sets that were implicated in viral responses and T-cell activity (ie, up-regulation of viral responses and T-cell activity were implicated in longer gestational length). Indeed, gene expression patterns that indicate down-regulation of adaptive immunity in women who deliver preterm was also observed in blood samples collected just before or during delivery.[31] Our transcription origin analyses did not detect differences in gene expression by gestational length that were attributable to T cells. But his pattern is possibly consistent with regulatory effect of dendritic cells on T cells, which include immune-suppressive subtypes that promote fetal tolerance and pregnancy maintenance.[65] Regardless, additional research, for example on isolated, specific cell types and of in vitro cross-cell activity during pregnancy, is required to evaluate these hypotheses.

These analyses are also novel in that associations between third trimester immune cell gene expression and birthweight were also considered. Independent of gestational length, lower birthweight was associated with up-regulation of genes regulated by the pro-inflammatory AP-1 transcription factor, but we observed no association for genes regulated by NF-kB. It is possible that this finding reflects a distinct contribution from cytokines or cellular activation processes that that are independent of the NF-kB pathway. Future research will be required to determine the biological processes leading to AP-1 activation, and to assess its significance in regulating the specific biological processes underlying birthweight and related pregnancy processes.

Interestingly, genes associated with both higher and lower birthweight were attributable to dendritic cells. This finding may reflect differences in dendritic cell activation (ie, down-regulation of basally expressed genes and simultaneous up-regulation of activation-associated genes), but it could also potentially reflect the activities of different dendritic cell subtypes and/or alterations in dendritic cell trafficking programs as noted above. For example, myeloid dendritic cells are conventional dendritic cells derived from monocytes that are activated by pathogens and cell death, whereas plasmacytoid or lymphoid dendritic cells display features consistent with lymphoid lineage and are involved in tolerance maintenance and viral defense.[61,63,66,67] As such, it is conceivable that increased activity of myeloid dendritic cells could be associated with processes related to lower birthweight, and plasmacytoid dendritic cells with those related to higher birthweight. Given that canonical dendritic cell activation markers were not associated with birthweight, the present data do not suggest that alterations in antigen-presenting function are likely to be involved. These analyses, however, do provide a rationale for examining dendritic cell function more closely in future research on the determinants of low birthweight. These analyses also implicated CD4+ and CD8+ T cells in contributing to the whole blood transcriptome associated with higher birthweight. These effects may reflect the well-known shift toward increased Th2 gene expression as the cellular immune system adapts to tolerate the fetal tissue graft.[38] Collectively, these results highlight the utility in considering the specific immune cells that could be driving differences in gene expression by birthweight, and point to additional avenues of research when understanding the immunological underpinnings of fetal development and growth.

There are several limitations to consider. First, our sample size is relatively small, consisting of 89 participants. However, our sample also consists of diverse, low-income women, traditionally underrepresented in health research. In addition, here we were able to control for confounds not consistently considered in the existing literature, that is infection and gestational age at sample collection. Second, gene expression data were derived from banked DBS, which tends to yield "noisier" transcriptome profiles than those derived from the larger blood volumes available from traditional venipuncture.[45] However, this "noise" implies that any true differences will be harder to detect, which underscores the strength of the particular pro-inflammatory metrics examined here and suggests that additional patterns may potentially be detected in future studies that use venipuncture blood samples. Third, there were also few cases of preterm birth or of clinically defined "low birthweight" in this sample, so all conclusions should be taken as directional trends within the normal range of variation at delivery. Although our results are broadly consistent with the expected association between immune gene activity and birth outcomes, these trends require further exploration in samples containing greater numbers of preterm and low birthweight infants. Finally, it is important

to note that this study was neither designed nor powered to identify specific individual gene transcripts that are associated with gestational length or birthweight; it was designed only to test specific a priori-defined hypotheses involving inflammation-related transcription factors and cells using an aggregate transcriptome-wide bioinformatics analysis that takes point estimates of differential gene expression as input (ie, there is no attempt to conduct statistical testing at the level of individual gene transcripts; statistical testing is conducted only at the level of transcription factor and/or cell identity composite scores derived up- and down-regulated gene sets). It is likely that additional transcriptional correlates of gestational length and birthweight exist that were not revealed by the present pre-specified hypothesis-testing analysis. Any such effects remain to be identified in future research using larger sample sizes and exploratory- or discovery-based statistical analyses.

In conclusion, increased pro-inflammatory gene expression in third trimester maternal blood samples was associated with both shorter gestational length and lower birthweight, via activity of the NF-kB and AP-1 transcription factors, respectively. Differences in immune cell gene expression by gestational length were attributed to monocytes and dendritic cells, and by birthweight to dendritic cells and T cells. These analyses contribute to our understanding of the pathways by which peripheral immune cell activity could increase risk for adverse pregnancy outcomes, that is shorter gestational length and low birthweight, and highlight the importance of examining cross-cell regulation and activity in future research.

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REFERENCES

1. Cunningham GF, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Song CY. *Williams Obstetrics*, 24th edn. New York, NY: McGraw Hill Professional; 2014.
2. Christian LM. Psychoneuroimmunology in pregnancy: immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. *Neurosci Biobehav Rev*. 2012;36(1):350-361.
3. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med*. 2006;11(5):317-326.
4. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. *Cell Mol Immunol*. 2014;11(6):571-581.
5. Denison FC, Roberts KA, Barr SM, Norman JE. Obesity, pregnancy, inflammation, and vascular function. *Reproduction*. 2010;140(3):373-385.
6. Park JS, Park CW, Lockwood CJ, Norwitz ER. Role of cytokines in preterm labor and birth. *Minerva Ginecol*. 2005;57(4):349-366.
7. Greig PC, Murtha AP, Jimmerson CJ, Herbert WN, Roitman-Johnson B, Allen J. Maternal serum interleukin-6 during pregnancy and during term and preterm labor. *Obstet Gynecol*. 1997;90(3):465-469.
8. Bowen JM, Chamley L, Keelan JA, Mitchell MD. Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition. *Placenta*. 2002;23(4):257-273.
9. Dizon-Townson DS. Preterm labour and delivery: a genetic predisposition. *Paediatr Perinat Epidemiol*. 2001;15(Suppl 2):57-62.
10. Murtha AP, Sinclair T, Hauser ER, Swamy GK, Herbert WN, Heine RP. Maternal serum cytokines in preterm premature rupture of membranes. *Obstet Gynecol*. 2007;109(1):121-127.
11. Holt R, Timmons BC, Akgul Y, Akins ML, Mahendroo M. The molecular mechanisms of cervical ripening differ between term and preterm birth. *Endocrinology*. 2011;152(3):1036-1046.
12. Ferguson KK, McElrath TF, Chen YH, Mukherjee B, Meeker JD. Longitudinal profiling of inflammatory cytokines and C-reactive protein during uncomplicated and preterm pregnancy. *Am J Reprod Immunol*. 2014;72(3):326-336.
13. Gargano JW, Holzman C, Senagore P, et al. Mid-pregnancy circulating cytokine levels, histologic chorioamnionitis and spontaneous preterm birth. *J Reprod Immunol*. 2008;79(1):100-110.
14. Sorokin Y, Romero R, Mele L, et al. Maternal serum interleukin-6, C-reactive protein, and matrix metalloproteinase-9 concentrations as risk factors for preterm birth <32 weeks and adverse neonatal outcomes. *Am J Perinatol*. 2010;27(8):631-640.
15. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol*. 1999;180(2 Pt 1):499-506.
16. Redman CW, Staff AC. Preeclampsia, biomarkers, syncytiotrophoblast stress, and placental capacity. *Am J Obstet Gynecol*. 2015;213(4 Suppl):S9.e1, S9-11.
17. Borzychowski AM, Sargent IL, Redman CW. Inflammation and preeclampsia. *Semin Fetal Neonatal Med*. 2006;11(5):309-316.
18. Kumarathasan P, Williams G, Bielecki A, et al. Characterization of maternal plasma biomarkers associated with delivery of small and large for gestational age infants in the MIREC study cohort. *PLoS ONE*. 2018;13(11):e0204863.
19. Kumarathasan P, Vincent R, Bielecki A, et al. Infant birthweight and third trimester maternal plasma markers of vascular integrity: the MIREC study. *Biomarkers*. 2016;21(3):257-266.
20. Arslan M, Yazici G, Erdem A, Erdem M, Ozturk Arslan E, Himmetoglu O. Endothelin 1 and leptin in the pathophysiology of intrauterine growth restriction. *Int J Gynaecol Obstet*. 2004;84(2):120-126.
21. Conde-Agudelo A, Papageorghiou AT, Kennedy SH, Villar J. Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis. *BJOG*. 2013;120(6):681-694.
22. Ernst GD, de Jonge LL, Hofman A, et al. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. *Am J Obstet Gynecol*. 2011;205(2):132.e1-132.e12.
23. Georgiou HM, Thio YS, Russell C, et al. Association between maternal serum cytokine profiles at 7–10 weeks' gestation and

birthweight in small for gestational age infants. *Am J Obstet Gynecol*. 2011;204(5):415.e1-415.e12.

24. Haddad R, Tromp G, Kuivaniemi H, et al. Human spontaneous labor without histologic chroioamnionitis is characterized by acute inflammation gene expression. *Am J Obstet Gynecol*. 2006;195(2):394.e1-394.e24.
25. Mayor-Lynn K, Toloubeydokhti T, Cruz AC, Chegini N. Expression profile of microRNAs and mRNAs in human placentas from pregnancies complicated by preeclampsia and preterm labor. *Reprod Sci*. 2011;18(1):46-56.
26. Gibbs I, Leavey K, Benton SJ, Grynspan D, Bainbridge SA, Cox BJ. Placental transcriptional and histologic subtypes of normotensive fetal growth restriction are comparable to preeclampsia. *Am J Obstet Gynecol*. 2018;220(1):110.e1-110.e21.
27. Thamotharan S, Chu A, Kempf K, et al. Differential microRNA expression in human placentas of term intra-uterine growth restriction that regulates target genes mediating angiogenesis and amino acid transport. *PLoS ONE*. 2017;12(5):e0176493.
28. Heng YJ, Pennell CE, McDonald SW, et al. Maternal whole blood gene expression at 18 and 28 weeks of gestation associated with spontaneous preterm birth in asymptomatic women. *PLoS ONE*. 2016;11(6):e0155191.
29. Paquette AG, Shynlova O, Kibschull M, et al. Comparative analysis of gene expression in maternal peripheral blood and monocytes during spontaneous preterm labor. *Am J Obstet Gynecol*. 2018;218(3):345.e1-345.e30.
30. Tarca AL, Romero R, Xu Z, et al. Targeted expression profiling by RNA-Seq improves detection of cellular dynamics during pregnancy and identifies a role for T cells in term parturition. *Sci Rep*. 2019;9(1):848.
31. Vora B, Wang A, Kosti I, et al. Meta-analysis of maternal and fetal transcriptomic data elucidates the role of adaptive and innate immunity in preterm birth. *Front Immunol*. 2018;9:993.
32. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005;102(43):15545-15550.
33. Tsang J, Vong J, Ji L, et al. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. *Proc Natl Acad Sci USA*. 2017;114(37):E7786-E7795.
34. Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol*. 2010;63(6):425-433.
35. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, Petraglia F. Inflammation and pregnancy. *Reprod Sci*. 2009;16(2):206-215.
36. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. *Immunol Today*. 1999;20(3):114-118.
37. Piccinni M-P. Role of immune cells in pregnancy. *Autoimmunity*. 2009;36(1):1-4.
38. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. *Am J Reprod Immunol*. 2010;63(6):601-610.
39. Sargent IL, Borzychowski AM, Redman CW. NK cells and human pregnancy–an inflammatory view. *Trends Immunol*. 2006;27(9):399-404.
40. Luppi P. How immune mechanisms are affected by pregnancy. *Vaccine*. 2003;21(24):3352-3357.
41. Cole SW, Hawkley LC, Arevalo JM, Cacioppo JT. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. *Proc Natl Acad Sci USA*. 2011;108(7):3080-3085.
42. Cole SW, Yan W, Galic Z, Arevalo J, Zack JA. Expression-based monitoring of transcription factor activity: the TELiS database. *Bioinformatics*. 2005;21(6):803-810.
43. Ramey SL, Schafer P, DeClerque JL, et al. The preconception stress and resiliency pathways model: a multi-level framework on maternal, paternal, and child health disparities derived by community-based participatory research. *Matern Child Health J*. 2015;19(4):707-719.
44. Dunkel Schetter C, Schafer P, Lanzi RG, et al. Shedding light on the mechanisms underlying health disparities through community participatory methods: the stress pathway. *Perspect Psychol Sci*. 2013;8(6):613-633.
45. McDade TW, Ross KM, Fried R, et al. Genome-wide profiling of RNA from dried blood spots: convergence with bioinformatics results derived from whole venous blood and peripheral blood mononuclear cells. *Biodemography Soc Biol*. 2016;62(2):182-197.
46. Mitchell AM, Porter K, Christian LM. Examination of the role of obesity in the association between childhood trauma and inflammation during pregnancy. *Health Psychol*. 2018;37(2):114-124.
47. Behrman RE, Butler AS. *Preterm Birth: Causes, Consequences, and Prevention*. Washington, DC: National Academy of Sciences; 2007.
48. Kuschner WG, D'Alessandro A, Wong H, Blanc PD. Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur Respir J*. 1996;9(10):1989-1994.
49. Imhof A, Koenig W. Alcohol inflammation and coronary heart disease. *Addict Biol*. 2003;8(3):271-277.
50. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine*. 2014;70:134-140.
51. Ross KM, Miller G, Culhane J, et al. Patterns of peripheral cytokine expression during pregnancy in two cohorts and associations with inflammatory markers in cord blood. *Am J Reprod Immunol*. 2016;76(5):406-414.
52. Cole SW, Galic Z, Zack JA. Controlling false-negative errors in microarray differential expression analysis: a PRIM approach. *Bioinformatics*. 2003;19(14):1808-1816.
53. Miller GE, Chen E, Sze J, et al. A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-kappaB signaling. *Biol Psychiatry*. 2008;64(4):266-272.
54. Fredrickson BL, Grewen KM, Coffey KA, et al. A functional genomic perspective on human well-being. *Proc Natl Acad Sci USA*. 2013;110(33):13684-13689.
55. Kutner M, Nachtsheim C, Neter J, Li W. *Applied Linear Statistics Models*, 5th edn. New York, NY: McGraw-Hill/Irwin; 2005.
56. Press WH, Teukolsky SA, Vetterling WT, Flannery BP. *Numerical Recipies in C: The Art of Scientific Computing*. New York, NY: Cambridge University Press; 2002.
57. Witten DM, Tibshirani R. A comparison of fold-change and the t-statistic for microarray data analysis. *Analysis*. 2007;1776:58-85.
58. Shi L, Jones WD, Jensen RV, et al. The balance of reproducibility, sensitivity, and specificity of lists of differentially expressed genes in microarray studies. *BMC Bioinformatics*. 2008;9(Suppl 9):S10.
59. Su AI, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA*. 2004;101(16):6062-6067.
60. Yoshimura T, Inaba M, Sugiura K, et al. Analyses of dendritic cell subsets in pregnancy. *Am J Reprod Immunol*. 2003;50(2):137-145.
61. Darmochwal-Kolarz D, Rolinski J, Tabarkiewicz J, et al. Myeloid and lymphoid dendritic cells in normal pregnancy and pre-eclampsia. *Clin Exp Immunol*. 2003;132(2):339-344.
62. Bachy V, Williams DJ, Ibrahim MA. Altered dendritic cell function in normal pregnancy. *J Reprod Immunol*. 2008;78(1):11-21.
63. Della Bella S, Giannelli S, Cozzi V, et al. Incomplete activation of peripheral blood dendritic cells during healthy human pregnancy. *Clin Exp Immunol*. 2011;164(2):180-192.
64. Yen J, Kocieda VP, Jing H, Ganea D. Prostaglandin E2 induces matrix metalloproteinase 9 expression in dendritic cells through two independent signaling pathways leading to activator protein 1 (AP-1) activation. *J Biol Chem*. 2011;286:38913-38923.

65. Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. *Immunology*. 2004;112(1):38-43.
66. Rissoan MC, Soumelis V, Kadowaki N, et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science*. 1999;283(5405):1183-1186.
67. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol*. 2005;23:275-306.

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