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Associations of Serum Inflammatory Biomarkers During Pregnancy With Placental Pathology and Placental Gene Expression at Delivery

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ABSTRACT

Problem: We sought to investigate whether maternal inflammatory cytokines during pregnancy are associated with histologic inflammatory or vascular lesions in the placenta and/or correlated with gene expression patterns in the placenta.

Method of Study: We leveraged data from a large randomized controlled trial (RCT) at a single site. Maternal serum was collected in the second and third trimesters, and a composite inflammatory score was created using five measured biomarkers (CRP, IL-6, IL-1ra, IL-10, and TNF-α). Placentas were collected at delivery for histological analysis and four major patterns of placental injury were characterized. Fresh small chorionic villous biopsies were collected for placental genome-wide mRNA profiling. Transcripts showing >2-fold differential expression over the 4-SD range of circulating inflammatory biomarkers were reported, adjusting for potential confounders.

Results: The primary analysis included 601 participants. A one standard deviation increase in the third-trimester inflammatory composite was associated with increased odds of chronic inflammation in the placenta (OR: 1.23, 95% CI 1.01, 1.51;). This was driven primarily by elevations in IL-10 (OR: 1.37; 99% CI: 1.06, 1.77). Higher maternal IL-10 in circulation was associated with bioinformatic indications of reduced pro-inflammatory gene regulation pathways in the placenta (AP1 decreased 25%, $p = 0.003$; NF-kB decreased 53%, $p = 0.003$) and indications of increased STAT family signaling pathways which mediate signaling through the IL-10 receptor (increased 73%, $p = 0.002$).

Conclusions: Our results indicate that elevated maternal circulating IL-10 during pregnancy is associated with chronic inflammatory lesions in the placenta at delivery. Additionally, higher levels of circulating IL-10 are associated with upregulated STAT signaling pathways in placental tissues.

1 | Introduction

Inflammatory cytokines in maternal circulation during pregnancy have been strongly associated with adverse outcomes including preterm birth [1–8], preeclampsia, and neurologic impairment in offspring [9, 10]. Furthermore, maternal race and socioeconomic status have recently been shown to be associated with inflammatory biomarkers during pregnancy [11], possibly related to structural racism [12, 13] and life course stressors [14]. Chronic, low-grade, subclinical maternal inflammatory status may affect placental development and fetal tolerance, and additionally, may lead to histologically visible placental inflammation. However, few studies have examined the placenta to test this hypothesis. Circulating maternal biomarkers of stress, particularly C-reactive protein (CRP) and Epstein Barr virus antibody have been shown to be associated with chronic inflammation (CI) characterized by lymphocytes and plasma cells in the placenta [14].

Although there is evidence that chronic placental inflammation is associated with preterm birth [15–17], the etiology of chronic placental inflammation remains unclear [18, 19] and its correlation with the maternal inflammatory milieu is understudied. It is also not clear how a chronic low-grade maternal inflammatory state might affect placental vascular development and contribute to maternal and/or fetal vascular disease in the placenta. There is evidence that CI affecting chorionic villi, such as chronic villitis of unknown etiology, can have secondary effects on the fetal vasculature of the villi [19], leading to thrombosis and avascular villi which can affect placental function. The combination of chronic placental inflammation and fetal vascular malperfusion (FVM) changes have been reported in a subset of patients with clinical features of preeclampsia [20], as well as with intrauterine growth restriction and neurological injury in neonates [19, 21].

In addition to circulating biomarkers, placental gene expression can provide a unique opportunity to understand upstream mechanistic pathways of placental injury, including upstream transcription factors potentially associated with alterations in placental function. To date, studies have examined transcription profiles in placental development, pregnancy complications, and exposures during pregnancy [22, 23], but to our knowledge, no studies have examined the relationship between differentially expressed genes, circulating maternal cytokines, and placental pathology.

Therefore, our primary aim was to investigate whether inflammatory biomarkers in maternal circulation during pregnancy are associated with histologically visible inflammatory and/or vascular lesions in the placenta. Then, our secondary aim was to identify inflammatory biomarkers most associated with placental lesions and test their associations with placental gene expression patterns.

2 | Materials and Methods

2.1 | Study Sample

This study leverages materials and data from the Psychosocial Intervention and Inflammation in Centering (PIINC) study. The PIINC study collected additional data from a randomized controlled trial (RCT) of group prenatal care (described elsewhere [24]) and enrolled 1256 participants at a single study site in Greenville, South Carolina. PIINC participants were enrolled between August 2018 and March 2020. Eligibility criteria included those 14–45 years old with a singleton pregnancy <24 weeks gestation, prenatal care initiation <21 weeks gestation, and English or Spanish proficiency. Those with known medical or pregnancy complications, including pregestational diabetes, chronic hypertension requiring medication, conditions requiring chronic immunosuppression, body mass index >50 kg/m^2, planned preterm delivery, and planned history-indicated cerclage, were excluded. Those with conditions that would preclude group prenatal care participation were also excluded (e.g., active tuberculosis, current incarceration, severe uncontrolled psychiatric illness). Participants were recruited and consented at their first prenatal care visit or during their dating ultrasound and randomly assigned to group or individual (traditional) prenatal care. Participants completed two blood draws, one in the second trimester (12–24 weeks gestation) and one in the third trimester (32–36 weeks gestation). Following delivery, placental specimens were collected for histologic review and analysis of mRNA expression.

2.2 | Circulating Cytokine Measures

Antecubital blood was drawn into a Serum-Separator Tube (Becton-Dickinson), which was centrifuged for 10 min at 1200 × *g* following venipuncture. The serum was harvested and divided into aliquots, then frozen at −80°C until the study ended. Five biomarkers of low-grade inflammation, most consistently related to both economic hardship and adverse pregnancy outcomes [25, 26], were measured in batch: CRP, and the cytokines interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1ra), interleukin10 (IL-10), and tumor necrosis factor-α (TNF-α). All were measured in triplicate using a multiplex immunoassay protocol on an automated microfluidic platform (Simple Plex, Protein Simple) [27]. The lower limit of detection for CRP was 1.24 pg/mL, and for the cytokines ranged from 0.11 pg/mL (IL-6) to 0.28 pg/mL (TNF-α). Across runs, the average intra-assay coefficients of variation for triplicate samples were 2.4% (CRP), 2.6% (IL-6), 2.1% (IL-1ra), 4.5% (IL-10), and 1.7% (TNF-α). The inter-assay coefficients of variation were 6.0% (CRP), 3.0% (IL-6), 3.2% (IL-1ra), 4.5% (IL-10), and 1.3% (TNF-α).

For analysis, biomarkers were log-transformed and standardized. To minimize false discovery, the five standardized biomarkers were summed to create a composite measure of inflammation for each trimester. Additionally, to evaluate individual biomarkers, the second and third-trimester biomarkers were averaged (Cronbach's alpha range: 0.62–0.82). Biomarkers with scores >3 standard deviations (SD) above the mean were considered outliers and excluded.

2.3 | Placenta Histologic Assessment

Following delivery, four large biopsies (2 in^3) of full-thickness placental tissue were collected from distinct areas of the placenta. In addition, a segment of umbilical cord (2 in) and a membrane roll (2 in × 7 in) were collected. Tissue specimens were fixed in formalin and shipped to a perinatal pathologist (LME)

for histologic review. Tissue specimens were processed into hematoxylin and eosin-stained slides and the following placental compartments were evaluated: membranes, umbilical cord, chorionic plate, basal plate, and villous parenchyma. For placentas that were not collected, medical records were reviewed and if the placenta was submitted for clinical pathological examination, placental histology slides from the clinical examination were obtained and reviewed by a single pathologist (LME).

Placental lesions were categorized into four major categories: acute inflammation (AI), CI, and two vascular: maternal vascular malperfusion (MVM), and FVM, using accepted, standardized, and current terminology [28, 29]. Briefly, AI included neutrophilic infiltration in either maternal and/or fetal compartments of acute: subchorionitis, chorionitis, chorioamnionitis, chorionic vasculitis, umbilical phlebitis, arteritis, funisitis. CI included the presence of lymphocytes, histiocytes, or plasma cells in placental tissue: chronic chorionic and/or chorioamnionitis of the membranes or chorionic plate, chronic villitis, chronic basal villitis, chronic deciduitis with plasma cells, chronic decidual perivasculitis, chronic intervillositis, and eosinophilic/T-cell vasculitis. MVM included decidual vascular lesions: fibrinoid necrosis/acute atherosis, muscularized basal plate arterioles, mural hypertrophy of membrane arterioles, basal decidual vascular thrombosis, and chorionic villous lesions: infarction, increased syncytial knots, villous agglutination, increased intervillous fibrin deposition, and distal villous hypoplasia. FVM included thrombosis of ≥1 fetal vessel (chorionic, stem villous, umbilical) and/or avascular villi. For AI, CI, and FVM, if any lesion within the pathology type was present then the pathology was considered present. Consistent with our prior work, a single lesion of MVM alone was not considered MVM [28]. Thus, the presence of MVM was determined based on the identification of ≥2 MVM lesions.

2.4 | Placental mRNA Expression

For gene expression, a small biopsy (0.5 cm^3) of fetal chorionic villous tissue was collected from four distinct cotyledons within 2 h of birth. Biopsies were rinsed in phosphate-buffered saline and stored in a stabilizing agent (AllProtect Tissue Reagent, Qiagen) at −80°C until the end of the study, at which time they were thawed, pooled, and then dissociated and homogenized on a gentleMACS (Miltenyi Biotec). Total RNA was extracted using Qiagen RNeasy Mini Kits. Genome-wide transcriptional profiling was conducted on 200 ng of total RNA. Samples were tested for suitable mass (≥10 ng by NanoDrop One spectrophotometry) and integrity (RNA Integrity Number ≥3 by Agilent TapeStation electrophoresis), converted to cDNA libraries using the Lexogen QuantSeq 3′ FWD enzyme system and sequenced in multiplex on an Illumina NovaSeq instrument, targeting >5 million single-strand 65-nt sequence reads per sample. Samples yielded an average of 5.3 million sequence reads (SD: 1.1 million), each mapped onto the GRCh38 human transcriptome sequence with STAR aligner [30]. Counts were standardized to transcripts per million mapped reads, floored at one normalized transcript per million mapped reads to minimize spurious variability, and log2-transformed for analysis.

As a secondary analysis, placental mRNA expression analysis was performed on a subset of the placental biopsies collected (n = 428). Samples were selected to include all those in the cohort with adverse pregnancy outcomes, defined as preterm delivery (<37 weeks gestation) or small for gestational age infant (SGA; birthweight <10th percentile for gestational age and sex [31]), and a random sample of the remaining biopsies.

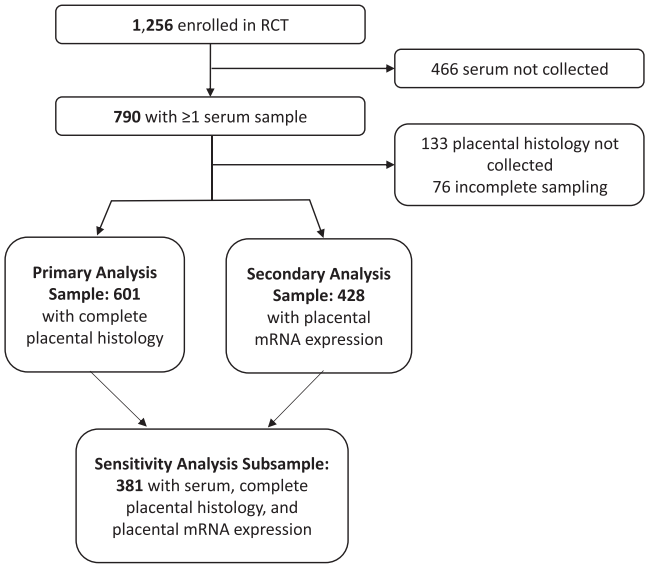

FIGURE 1 | Study inclusion flowchart.

2.5 | Statistical Analysis

Associations between placental inflammatory and/or vascular pathology (AI, CI, FVM, MVM) and composite inflammatory biomarkers during pregnancy were estimated using logistic regression. Separate models evaluated each placental pathology type. Models were adjusted for gestational age at blood draw, maternal age, race/ethnicity, body mass index, infant sex, and prenatal care type (RCT assignment). A p value <0.05 was considered statistically significant. Models did not include adjustment for pathology co-occurrence. However, in a post-hoc analysis, we additionally evaluated whether any statistically significant associations between placental pathology and composite inflammatory biomarkers were influenced by the presence of co-occurring pathology. This analysis was parameterized using logistic regression with interaction terms between the composite inflammatory biomarker and the co-occurring pathology of interest to evaluate whether the association differed based on co-occurring pathology. In a follow-up analysis, we tested associations between placental pathology and individual biomarkers (averaged across trimesters). To account for multiple testing in this analysis, a p value <0.01 was used to determine statistical significance (0.05/5 biomarkers) and 99% confidence intervals (CI) were reported. Analyses were conducted using SAS version 9.4 (SAS Institute INC., Cary, North Carolina).

Any individual inflammatory biomarker significantly associated with placental pathology in the primary analysis was included in the secondary analysis of inflammatory biomarkers and placental gene expression. Placental genome-wide mRNA profiles were analyzed to identify transcripts showing >2-fold differential expression over the 4-SD range of serum inflammatory

TABLE 1 | Descriptive characteristics for the cytokine, pathology, and mRNA study samples.

N (%) or mean ± SD	Primary analysis Placental pathology/cytokine (*n* = 601)	Secondary analysis mRNA/cytokine (*n* = 428)
Demographic characteristics		
Maternal age, years	25.3 ± 5.5	25.4 ± 5.5
Race		
Black	208 (34.6)	149 (34.8)
Hispanic	138 (23.0)	95 (22.2)
White	234 (38.9)	163 (38.1)
Other	21 (3.5)	21 (4.9)
Education, high school, or more	412 (71.9)	300 (73.4)
Employed, full, or part-time	320 (56.3)	223 (55.5)
Married	410 (75.7)	295 (76.2)
Annual household income[a]		
<$10 000	110 (27.6)	80 (27.9)
$10 000–$19 999	163 (40.8)	124 (43.2)
$20 000–$49 999	120 (30.1)	77 (26.8)
>$50 000	6 (1.5)	6 (2.1)
Nulliparous	263 (43.8)	191 (44.6)
Pre-pregnancy BMI, kg/m^2	29.2 ± 7.3	29.4 ±7.3
Pregnancy characteristics		
Gestational age at 2nd-trimester serum	16.2 ± 2.7	16.3 ± 2.9
Gestational age at 3rd-trimester serum	34.3 ± 1.6	34.3 ± 1.6
Smoked during pregnancy	112 (19.0)	85 (20.2)
Preterm birth (<37 weeks gestation)	66 (11.0)	64 (14.9)
SGA birth	73 (12.2)	80 (18.7)
Infant sex, male	284 (47.4)	203 (47.4)
Mode of delivery, C-section	163 (27.2)	117 (27.3)
Placenta characteristics		
Acute inflammation	359 (59.7)	256 (60.1)
Chronic inflammation	294 (48.9)	202 (47.4)
Maternal vascular malperfusion	158 (26.3)	126 (29.6)
Fetal vascular malperfusion	96 (16.0)	65 (15.3)
Inflammatory biomarkers[b]		
CRP, mg/L	16.2 ± 16.8	15.9 ± 16.4
IL-10, pg/mL	2.2 ± 1.4	2.2 ± 1.5
IL-1Ra, pg/mL	554.2 ± 273.9	555.4 ± 269.0
IL-6, pg/mL	3.4 ± 6.0	3.6 ± 7.0
TNF-α, pg/mL	10.0 ± 2.4	10.0 ± 2.5

Abbreviations: BMI, body mass index; SD, standard deviation.

[a]Missing 202 in the primary analysis sample and 141 in the secondary analysis sample.

[b]Average of 2nd- and 3rd-trimester serum measures.

TABLE 2 | Descriptive characteristics for the study sample ($n = 1256$), stratified by inclusion in the primary analysis ($n = 601$) versus excluded due to serum and/or placental pathology not collected ($n = 655$).

N (%) or mean ± SD	Analytic sample ($n = 601$)	Excluded ($n = 655$)	*p* value
Demographic characteristics			
Maternal age, years	25.3 ± 5.5	24.8 ± 5.1	0.06
Race			0.08
Black	208 (34.6)	223 (34.0)	
Hispanic	138 (23.0)	119 (18.2)	
White	234 (38.9)	279 (42.6)	
Other	21 (3.5)	34 (5.2)	
Education, high school, or more	412 (71.9)	469 (74.8)	0.26
Employed, full or part-time	320 (56.3)	322 (52.9)	0.44
Married	410 (75.7)	432 (75.7)	0.99
Annual household income[a]			0.01
<$10 000	110 (27.6)	142 (31.4)	
$10 000–$19 999	163 (40.8)	207 (45.8)	
$20 000–$49 999	120 (30.1)	91 (20.1)	
>$50 000	6 (1.5)	12 (2.7)	
Nulliparous	263 (43.8)	299 (45.6)	0.50
Pre-pregnancy BMI, kg/m^2	29.2 ± 7.3	28.9 ±7.4	0.50
RCT assignment, group prenatal care	292 (48.6)	317 (48.4)	0.95
Pregnancy characteristics			
Gestational age at 2nd-trimester serum	16.2 ± 2.7		
Gestational age at 3rd-trimester serum	34.3 ± 1.6		
Smoked during pregnancy	112 (19.0)	118 (18.6)	0.85
Preterm birth	66 (11.0)	39 (6.1)	<0.01
SGA birth	73 (12.2)	80 (13.1)	0.64
Infant sex, male	284 (47.4)	344 (53.8)	0.02
Placenta characteristics			
Acute inflammation	359 (59.7)		
Chronic inflammation	294 (48.9)		
Maternal vascular malperfusion	158 (26.3)		
Fetal vascular malperfusion	96 (16.0)		

Abbreviations: BMI, body mass index; RCT, randomized controlled trial; SD, standard deviation.
[a]Missing for 405 (32.2%).

biomarkers, adjusting for potential confounders (maternal age, body mass index at first prenatal visit, race/ethnicity, infant sex, gestational age at delivery, and mode of delivery). These transcripts served as input into the Transcription Element Listening System (TELiS) to complete bioinformatic analysis of transcription factors [32], focusing on a priori-defined transcription control pathways involved in regulating inflammation, hypoxia, interferon response or neural signaling, as represented by TRANSFAC position-specific weight matrices for NF-κB, AP1, STAT, HIF, IRF, and CREB transcription factors. As a sensitivity analysis, we replicated the primary analysis in the subset of study participants that are included in both analyses (i.e., have complete data on inflammatory biomarkers, placental pathology, and placental gene expression).

3 | Results

3.1 | Inflammatory Biomarkers and Placental Pathology

The analysis included 601 participants who completed ≥1 blood draw and whose placentas were fully and adequately sampled for histology (Figure 1). Of these, 381 participants also had mRNA

TABLE 3 | Associations between biomarkers of inflammation in serum during pregnancy and placental histology ($n = 601$).

	Acute inflammation		Chronic inflammation		Maternal vascular malperfusion		Fetal vascular malperfusion	
	Unadjusted OR (CI)	**Adjusted[a] OR (CI)**	**Unadjusted OR (CI)**	**Adjusted[a] OR (CI)**	**Unadjusted OR (CI)**	**Adjusted[a] OR (CI)**	**Unadjusted OR (CI)**	**Adjusted[a] OR (CI)**
Composite[b]								
2nd trimester	1.00 (0.83, 1.20)	1.08 (0.85, 1.39)	1.09 (0.91, 1.30)	1.09 (0.86, 1.39)	1.18 (0.96, 1.45)	1.24 (0.95, 1.61)	0.99 (0.77, 1.27)	0.98 (0.71, 1.36)
3rd trimester	1.02 (0.87, 1.21)	1.07 (0.88, 1.31)	1.17 (0.99, 1.37)	**1.23 (1.01, 1.51)**	1.13 (0.94, 1.36)	1.10 (0.88, 1.38)	1.14 (0.91, 1.42)	1.21 (0.94, 1.58)
Individual biomarkers (average)[c]								
CRP	0.98 (0.77, 1.24)	1.03 (0.78, 1.36)	1.20 (0.95, 1.52)	1.23 (0.94, 1.62)	1.16 (0.89, 1.52)	1.15 (0.84, 1.57)	0.95 (0.69, 1.29)	0.88 (0.62, 1.27)
IL-10	1.02 (0.80, 1.30)	1.02 (0.80, 1.30)	**1.38 (1.08, 1.77)**	**1.37 (1.06, 1.77)**	1.17 (0.90, 1.52)	1.15 (0.88, 1.51)	1.16 (0.85, 1.57)	1.22 (0.90, 1.66)
IL-1Ra	0.94 (0.75, 1.18)	0.94 (0.70, 1.26)	0.94 (0.75, 1.17)	0.87 (0.66, 1.17)	1.21 (0.95, 1.56)	1.22 (0.88, 1.69)	0.85 (0.62, 1.16)	0.77 (0.51, 1.16)
IL-6	1.10 (0.87, 1.39)	1.19 (0.89, 1.59)	1.16 (0.92, 1.46)	1.19 (0.91, 1.56)	1.13 (0.88, 1.46)	1.09 (0.81, 1.46)	1.21 (0.91, 1.61)	1.20 (0.86, 1.66)
TNF-α	1.01 (0.80, 1.27)	1.06 (0.82, 1.37)	1.10 (0.88, 1.38)	1.11 (0.86, 1.43)	1.17 (0.90, 1.50)	1.19 (0.89, 1.58)	1.18 (0.87, 1.60)	1.26 (0.90, 1.77)

Note: Bold values represent confidence intervals that do not cross 1 and thus are statistically signficant.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Adjusted for gestational age at blood draw, maternal age, body mass index, infant sex, race, and prenatal care type.

[b]95% CI presented for composite measures.

[c]99% CI presented for individual biomarkers to account for multiple testing.

TABLE 4 | Prevalence of individual chronic inflammatory lesions and levels of IL-10 by lesion presence ($n = 601$).

Chronic inflammatory lesion	*N* (%)		IL-10, pg/mL[a]		*p* value
	Lesion present	Lesion absent	Lesion present	Lesion absent	
Chronic villitis	47 (7.8)	554 (92.2)	2.3 (1.2)	2.2 (1.4)	0.55
Chronic basal villitis	69 (11.5)	532 (88.5)	2.6 (2.8)	2.1 (1.1)	0.15
Chronic deciduitis with plasma cells	262 (43.6)	339 (56.4)	2.3 (1.4)	2.1 (1.4)	0.04
Chronic marginating choriodeciduitis	38 (6.3)	563 (93.7)	2.4 (0.8)	2.2 (1.4)	0.16

[a]Average of 2nd- and 3rd-trimester serum measures, presented as mean (standard deviation).

data. The mean age of the 601 participants was 23.5 years (SD: 5.5), the sample was racially and ethnically diverse, and 19% reported smoking during pregnancy (Table 1). 11.0% delivered a preterm infant and 12.2% delivered a SGA infant. The mean gestational age at serum collections was 16.2 weeks for the second-trimester sample (SD: 2.7) and 34.3 weeks for the third-trimester sample (SD: 1.6) (Table 1). The most prevalent placental pathology category was AI (59.7%), followed by CI (48.9%), MVM (26.3%), and FVM (16.0%). Amongst all the PIINC participants ($N = 1256$), those excluded from the primary analysis for lack of serum or placental samples had generally lower household income and were less likely to deliver preterm (Table 2).

In the adjusted model, a one SD increase in the third-trimester inflammatory composite was associated with increased odds of CI in the placenta (odds ratio [OR]: 1.23, 95% CI: 1.01, 1.51; Table 3). This was driven primarily by elevations in IL-10 (OR: 1.37; 99% CI: 1.06, 1.77). The observed association between the third-trimester inflammatory composite and CI also did not differ by the presence of co-occurring lesions of AI, MVM, or FVM (all interaction p values > 0.10). In an exploratory analysis evaluating differences in IL-10 by individual types of CI lesions, IL-10 levels were consistently higher among placentas with the CI lesions of interest (Table 4) The inflammatory composite and individual biomarkers were not significantly associated with any other placental pathology (AI, FVM, or MVM) in adjusted analyses. Results were consistent in the sensitivity analysis restricted to the subset of 381 included in both the pathology (primary analysis) and mRNA (secondary analysis) samples (Table 5).

3.2 | Inflammatory Biomarkers and Placental Gene Expression

For the secondary analysis, maternal circulating biomarkers and placental mRNA data were available for 428 participants, the vast majority ($n = 381$, 89%) of whom also had complete placental histology and were included in the primary analysis. Demographic and placental characteristics of those included in the secondary analysis were similar to the primary analysis sample (Table 1), except that, as expected, due to sampling selection, those in the secondary analysis had a higher prevalence of preterm birth (14.9%) and SGA infant (18.7%).

In gene expression analyses, 180 transcripts were differentially expressed in placental tissues as a function of circulating IL-10 levels (146 upregulated, 34 downregulated). See Table S1. Furthermore, the TELiS analysis revealed that higher maternal IL-10 in circulation was associated with bioinformatic indications of reduced activity in key pro-inflammatory signaling pathways in placentas (AP1 decreased 25%, $p = 0.003$; NF-κB decreased 53%, $p = 0.003$) and indications of higher activity of the STAT family signaling pathways that mediate signaling through the IL-10 receptor (increased 73%, $p = 0.002$) (Figure 2). There were no differences in pathways related to tissue hypoxia, immune response, or neural signaling.

4 | Conclusions

Our results indicate that elevated maternal inflammatory biomarkers in circulation, particularly IL-10, are associated with the development of chronic inflammatory lesions in the placenta, detectable at delivery. Additionally, relatively higher levels of circulating IL-10 in maternal blood in pregnancy are associated with differential gene expression in placental tissues at delivery, with promoter sequence-based bioinformatic analyses implicating AP-1, STAT, and NF-κB in particular. Other placental pathologies did not associate with the circulating inflammatory cytokines interrogated in this study. In particular, acute inflammatory lesions in the placenta at delivery were not associated with these inflammatory biomarkers. This might be explained by the fact that blood for biomarker analysis was collected many weeks prior to delivery and acute inflammatory lesions usually develop in the hours to days prior to delivery.

IL-10 is an anti-inflammatory, immunoregulatory cytokine that is produced by immune cells such as monocytes/histiocytes, T-helper lymphocytes, mast cells, and regulatory T cells [33]. Its expression can be triggered by immune activation from endotoxin, TNF-alpha, and catecholamines [34]. IL-10's main function is to tamp down the immune response by suppressing MHC class II expression and CD80/86 expression [34], thereby reducing inflammation. This response has an important role in maintaining immune tolerance [34] and dysregulation of the IL-10 response has been described in autoimmune diseases, COVID-19 infection, and cancer [35]. In pregnancy, dysregulation of the IL-10 pathway has been described in preeclampsia, preterm birth, miscarriage, and fetal growth restriction [36–40]. Our findings of a correlation between higher levels of IL-10, an anti-inflammatory cytokine, and the presence of histologic CI in the placenta seem counterintuitive at face value. However, elevations in serum IL-10 without a concomitant elevation in other pro-inflammatory cytokines and in the face of chronic inflammatory infiltrates may

TABLE 5 | Sensitivity analysis of associations between biomarkers of inflammation in serum during pregnancy and placental histology among the subset with serum cytokines, placental histology, and placental mRNA expression ($n = 381$).

	Acute inflammation		Chronic inflammation		Maternal vascular malperfusion		Fetal vascular malperfusion	
	Unadjusted OR (CI)	Adjusted[a] OR (CI)	Unadjusted OR (CI)	Adjusted[a] OR (CI)	Unadjusted OR (CI)	Adjusted[a] OR (CI)	Unadjusted OR (CI)	Adjusted[a] OR (CI)
Composite[b]								
2nd trimester	0.96 (0.77, 1.21)	1.03 (0.76, 1.40)	1.06 (0.84, 1.32)	1.05 (0.78, 1.41)	1.21 (0.97, 1.52)	1.32 (0.98, 1.78)	0.95 (0.70, 1.31)	0.86 (0.56, 1.31)
3rd trimester	1.02 (0.84, 1.24)	1.12 (0.88, 1.41)	**1.27 (1.05, 1.54)**	**1.34 (1.06, 1.69)**	**1.22 (1.01, 1.48)**	1.21 (0.96, 1.52)	0.97 (0.74, 1.27)	0.99 (0.72, 1.36)
Individual biomarkers (average)[c]								
CRP	0.97 (0.73, 1.29)	1.04 (0.75, 1.46)	1.27 (0.95, 1.68)	1.28 (0.91, 1.80)	1.21 (0.92, 1.61)	1.21 (0.87, 1.68)	0.92 (0.63, 1.35)	0.88 (0.57, 1.38)
IL-10	0.97 (0.73, 1.30)	0.96 (0.71, 1.30)	**1.40 (1.03, 1.90)**	**1.37 (1.01, 1.88)**	1.24 (0.92, 1.67)	1.22 (0.90, 1.66)	0.96 (0.64, 1.46)	1.02 (0.68, 1.54)
IL-1Ra	0.90 (0.68, 1.19)	0.91 (0.64, 1.31)	1.00 (0.76, 1.32)	0.99 (0.69, 1.41)	1.19 (0.90, 1.58)	1.16 (0.81, 1.66)	0.83 (0.55, 1.26)	0.74 (0.44, 1.25)
IL-6	1.12 (0.85, 1.48)	1.26 (0.89, 1.79)	1.29 (0.97, 1.71)	1.34 (0.96, 1.87)	1.03 (0.79, 1.35)	0.98 (0.72, 1.33)	1.10 (0.77, 1.58)	1.10 (0.74, 1.65)
TNF-α	0.96 (0.72, 1.28)	1.03 (0.75, 1.42)	1.10 (0.83, 1.45)	1.09 (0.80, 1.50)	1.26 (0.94, 1.68)	1.26 (0.91, 1.73)	0.94 (0.63, 1.41)	0.96 (0.62, 1.50)

Note: Bold values represent confidence intervals that do not cross 1 and thus are statistically signficant.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Adjusted for gestational age at blood draw, maternal age, body mass index, infant sex, race, and prenatal care type.

[b]95% CI presented for composite measures.

[c]99% CI presented for individual biomarkers to account for multiple testing.

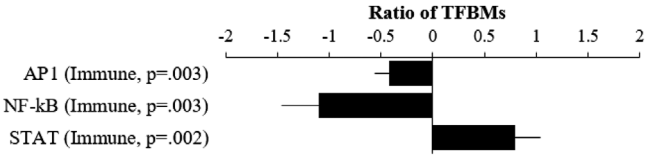

FIGURE 2 | Results of TELiS analysis. The X-axis displays the log2 ratio of transcription factor-binding motifs (TFBMs) in core promoter sequences of differentially expressed genes with >2-fold variation in expression over a 4-SD range of IL-10 levels in maternal serum. Error bars reflect the standard error.

be a sign of the attempt to clear up a previously more robust inflammatory response or may represent dysregulation of the inflammatory response leading to persistent CI.

CI in the placenta is defined as the presence of an abnormal histiocytic, lymphocytic, or plasma cell response in any placental compartment. The classic chronic inflammatory placental lesion is chronic villitis of unknown etiology (CVUE) which is believed by many to represent an abnormal immune attack on fetal chorionic villous cells by maternal immune cells [19, 41]. Immunohistochemical studies of CVUE have confirmed that lesions are composed of histiocytes, regulatory T-cells and that phosphorylated STAT-1 protein is expressed on trophoblast cells, suggesting activation of the STAT pathway [42, 43]. In our analysis, higher levels of IL-10 in maternal serum were associated with (1) activation of the JAK-STAT pathway in placental tissues at delivery and (2) histologic chronic placental inflammation in the placenta at delivery. It is worthy of note, that in this study chronic inflammatory lesions in the placenta included not only the classic CVUE, but also chronic inflammatory infiltrates in other compartments of the placenta such as the membranes, chorionic plate, decidua, and intervillous space. Interestingly, our gene expression analysis also showed that higher IL-10 levels were associated with down-regulation of two major inflammatory pathways AP1 and NF-κB, which is likely the effect of IL-10 inhibition on these inflammatory pathways. IL-10 activation is known to inhibit NF-κB signaling [34] and the upregulation of the STAT pathway has been shown to have inhibitory effects on the transcription of multiple genes involved in the inflammatory cascade including the genes of cytokines such as TNF-α, IL-1, and IL-6, genes involved in cell-mediated immunity such as MHC class II molecules and CD80/CD86, and IL-4-inducible genes such CD23 and IL-1 receptor [44].

Interestingly, many of the studies of IL-10 in poor pregnancy outcomes show that a deficiency of IL-10 is associated with adverse outcomes due to the loss of immunoregulation and the presence of a proinflammatory state at the maternal-fetal interface [34, 45, 46]. Our data, which was not concentrated on adverse pregnancy outcomes, indicate that high IL-10 levels in maternal serum are associated with chronic placental inflammation at delivery and both up and down-regulation of specific inflammatory gene transcription pathways expected in an IL-10 upregulated state. Therefore, our data indicate that the histologic appearance of chronic placental inflammation is correlated with a state of IL-10 upregulation, and suggest that circulating IL-10 in maternal serum may be a marker of chronic placental inflammation.

This study's strengths include its large sample size and the correlation of placental pathology as assessed by histology, maternal serum cytokines at two time points during pregnancy, and placental gene expression. To our knowledge, this is the first study to combine these three modalities. Furthermore, this is a prospectively collected sample and thus not subject to the biases inherent in studies restricted to placentas with clinical indications for pathologic examination. There was also a comprehensive pathologic analysis performed by an expert perinatal pathologist using current terminology and classifications. Relatedly, our prevalence of acute (59.7%) and chronic (48.9%) inflammation is different from that reported by Romero et al. (2018), who found rates of 42.3% and 29.9%, respectively in a cohort of 944 term pregnancies without obstetric complications [47]. Importantly, the higher prevalence in our sample may reflect the inclusion of obstetric complications, such as preterm birth. Additionally, our prior research suggests that CI may be under-identified, particularly by general surgical pathologists [48].

Despite these strengths, our analysis does have weaknesses. Placental pathology and serum cytokines were not available for all study participants, and thus selection bias may be a limitation. However, there were minimal sociodemographic and clinical differences in the included and excluded participants. Although preterm births were more likely to be included in our analytic sample, the prevalence of preterm births in the analytic sample (11%) remained consistent with national statistics. Further, our results were consistent in a sensitivity analysis among participants with complete serum and placental data. Another limitation of our analysis is the inability to assess the directionality of the associations. Although IL-10 was assessed during pregnancy and chronic placental inflammation was assessed following delivery, chronic inflammatory lesions may have developed earlier in pregnancy. We cannot be certain if higher IL-10 is the cause or effect of chronic placental inflammation. Additionally, IL-10 had the lowest concentration of the cytokines evaluated. However, the low inter- and intra-assay coefficients of variation (both 4.5%) indicate reliable and consistent quantification. Further, minor fluctuations in measurement would likely result in random error and bias results toward the null. Finally, we did not have detailed information on labor characteristics that could influence inflammatory processes [49]. However, our models of inflammatory cytokines and placental gene expression included mode of delivery as a proxy to account for potential differences.

In summary, we show that elevated maternal serum inflammatory biomarkers in the second and third trimester of pregnancy, particularly IL-10, are associated with the development of chronic inflammatory lesions in the placenta, detectable at delivery and that relatively higher levels of circulating IL-10 in maternal blood associate with upregulated STAT signaling pathways in placental tissues at delivery. Caution is needed in interpreting this correlation, but these data are consistent with the hypothesis that maternal IL-10 serum levels are related to placental CI.

Disclosure

The funders had no role in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The study conformed to the US Federal Policy for the Protection of Human Subjects.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Deidentified study data will be available publicly on the NICHD/DASH Data and Specimen Hub (https://dash.nichd.nih.gov/) five years after study completion (March 2028). Prior to that time, researchers with a methodologically sound proposal can direct inquiries to aborders@northshore.org to gain access to the study protocol, informed consent forms, deidentified data, data dictionaries and the analytic plan. Requestors will need to sign a data access agreement.

References

1. L. S. Keenan-Devlin, M. Caplan, A. Freedman, et al., "Using Principal Component Analysis to Examine Associations of Early Pregnancy Inflammatory Biomarker Profiles and Adverse Birth Outcomes," *American Journal of Reproductive Immunology* 86, no. 6 (2021): e13497, https://doi.org/10.1111/aji.13497.

2. M. E. Coussons-Read, M. Lobel, J. C. Carey, et al., "The Occurrence of Preterm Delivery Is Linked to Pregnancy-Specific Distress and Elevated Inflammatory Markers Across Gestation," *Brain, Behavior, and Immunity* 26, no. 4 (2012): 650–659, https://doi.org/10.1016/j.bbi.2012.02.009.

3. D. S. Dizon-Townson, "Preterm Labour and Delivery: A Genetic Predisposition," *Paediatric and Perinatal Epidemiology* 15, no. s2 (2001): 57–62, https://doi.org/10.1046/j.1365-3016.2001.00008.x.

4. V. Lohsoonthorn, C. Qiu, and M. A. Williams, "Maternal Serum C-Reactive Protein Concentrations in Early Pregnancy and Subsequent Risk of Preterm Delivery," *Clinical Biochemistry* 40, no. 5 (2007): 330–335, https://doi.org/10.1016/j.clinbiochem.2006.11.017.

5. F. Lucaroni, L. Morciano, G. Rizzo, et al., "Biomarkers for Predicting Spontaneous Preterm Birth: An Umbrella Systematic Review," *Journal of Maternal-Fetal & Neonatal Medicine* 31, no. 6 (2018): 726–734, https://doi.org/10.1080/14767058.2017.1297404.

6. A. P. Murtha, T. Sinclair, E. R. Hauser, G. K. Swamy, W. N. P. Herbert, and R. P. Heine, "Maternal Serum Cytokines in Preterm Premature Rupture of Membranes," *Obstetrics & Gynecology* 109, no. 1 (2007): 121–127, https://doi.org/10.1097/01.AOG.0000250474.35369.12.

7. W. Pitiphat, M. W. Gillman, K. J. Joshipura, P. L. Williams, C. W. Douglass, and R.-E. JW, "Plasma C-Reactive Protein in Early Pregnancy and Preterm Delivery," *American Journal of Epidemiology* 2005;162(11):1108–1113, https://doi.org/10.1093/aje/kwi323.

8. R. Romero, J. C. Grivel, A. L. Tarca, et al., "Evidence of Perturbations of the Cytokine Network in Preterm Labor," *American Journal of Obstetrics and Gynecology* 213, no. 6 (2015): 836.e1–836.e18, https://doi.org/10.1016/j.ajog.2015.07.037.

9. K. K. Ferguson, T. F. McElrath, Y. Chen, B. Mukherjee, and J. D. Meeker, "Longitudinal Profiling of Inflammatory Cytokines and C-Reactive Protein During Uncomplicated and Preterm Pregnancy," *American Journal of Reproductive Immunology* 72, no. 3 (2014): 326–336, https://doi.org/10.1111/aji.12265.

10. N. M. Jiang, M. Cowan, S. N. Moonah, and W. A. Petri, "The Impact of Systemic Inflammation on Neurodevelopment," *Trends in Molecular Medicine* 24, no. 9 (2018): 794–804, https://doi.org/10.1016/j.molmed.2018.06.008.

11. L. S. Keenan-Devlin, B. P. Smart, W. Grobman, et al., "The Intersection of Race and Socioeconomic Status Is Associated With Inflammation Patterns During Pregnancy and Adverse Pregnancy Outcomes," *American Journal of Reproductive Immunology* 87, no. 3 (2022): e13489, https://doi.org/10.1111/aji.13489.

12. A. D. Richman, "Concurrent Social Disadvantages and Chronic Inflammation: The Intersection of Race and Ethnicity, Gender, and Socioeconomic Status," *Journal of Racial and Ethnic Health Disparities* 5, no. 4 (2018): 787–797, https://doi.org/10.1007/s40615-017-0424-3.

13. I. Stepanikova, L. B. Bateman, and G. R. Oates, "Systemic Inflammation in Midlife: Race, Socioeconomic Status, and Perceived Discrimination," *American Journal of Preventive Medicine* 52, no. 1 (2017): S63–S76, https://doi.org/10.1016/j.amepre.2016.09.026.

14. L. Ernst, W. Grobman, K. Wolfe, et al., "Biological Markers of Stress in Pregnancy: Associations With Chronic Placental Inflammation at Delivery," *American Journal of Perinatology* 30, no. 07 (2012): 557–564, https://doi.org/10.1055/s-0032-1329187.

15. N. Edmondson, A. Bocking, G. Machin, R. Rizek, C. Watson, and S. Keating, "The Prevalence of Chronic Deciduitis in Cases of Preterm Labor Without Clinical Chorioamnionitis," *Pediatric and Developmental Pathology* 12, no. 1 (2009): 16–21, https://doi.org/10.2350/07-04-0270.1.

16. C. J. Kim, R. Romero, J. P. Kusanovic, et al., "The Frequency, Clinical Significance, and Pathological Features of Chronic Chorioamnionitis: A Lesion Associated With Spontaneous Preterm Birth," *Modern Pathology* 23, no. 7 (2010): 1000–1011, https://doi.org/10.1038/modpathol.2010.73.

17. J. Lee, R. Romero, Y. Xu, et al., "A Signature of Maternal Anti-Fetal Rejection in Spontaneous Preterm Birth: Chronic Chorioamnionitis, Anti-Human Leukocyte Antigen Antibodies, and C4d," *PLoS ONE* 6, no. 2 (2011): e16806, https://doi.org/10.1371/journal.pone.0016806.

18. L. M. Ernst, C. Bockoven, A. Freedman, et al., "Chronic Villitis of Unknown Etiology: Investigations Into Viral Pathogenesis," *Placenta* 107 (2021): 24–30, https://doi.org/10.1016/j.placenta.2021.02.020.

19. R. W. Redline, "Villitis of Unknown Etiology: Noninfectious Chronic Villitis in the Placenta," *Human Pathology* 38, no. 10 (2007): 1439–1446, https://doi.org/10.1016/j.humpath.2007.05.025.

20. A. A. Freedman, S. Suresh, and L. M. Ernst, "Patterns of Placental Pathology Associated With Preeclampsia," *Placenta* 139 (2023): 85–91, https://doi.org/10.1016/j.placenta.2023.06.007.

21. R. W. Redline, "Severe Fetal Placental Vascular Lesions in Term Infants With Neurologic Impairment," *American Journal of Obstetrics and Gynecology* 192, no. 2 (2005): 452–457, https://doi.org/10.1016/j.ajog.2004.07.030.

22. J. Saben, Y. Zhong, S. McKelvey, et al., "A Comprehensive Analysis of the human Placenta Transcriptome," *Placenta* 35, no. 2 (2014): 125–131, https://doi.org/10.1016/j.placenta.2013.11.007.

23. H. E. J. Yong and S. Y. Chan, "Current Approaches and Developments in Transcript Profiling of the Human Placenta," *Human Reproduction Update* 26, no. 6 (2020): 799–840, https://doi.org/10.1093/humupd/dmaa028.

24. A. H. Crockett, L. Chen, E. C. Heberlein, et al., "Group vs Traditional Prenatal Care for Improving Racial Equity in Preterm Birth and Low Birthweight: The Centering and Racial Disparities Randomized Clinical Trial Study," *American Journal of Obstetrics and Gynecology* 227, no. 6 (2022): 893.e1–893.e15, https://doi.org/10.1016/j.ajog.2022.06.066.

25. P. H. Lam, J. J. Chiang, E. Chen, and G. E. Miller, "Race, Socioeconomic Status, and Low-Grade Inflammatory Biomarkers Across the Lifecourse: A Pooled Analysis of Seven Studies," *Psychoneuroendocrinology* 123 (2021): 104917, https://doi.org/10.1016/j.psyneuen.2020.104917.

26. J. Gomes, F. Au, A. Basak, S. Cakmak, R. Vincent, and P. Kumarathasan, "Maternal Blood Biomarkers and Adverse Pregnancy Outcomes: A Systematic Review and Meta-Analysis," *Critical Reviews in Toxicology* 49, no. 6 (2019): 461–478, https://doi.org/10.1080/10408444.2019.1629873.

27. P. Aldo, G. Marusov, D. Svancara, J. David, and G. Mor, "Simple Plex ™ : A Novel Multi-Analyte, Automated Microfluidic Immunoas-

say Platform for the Detection of Human and Mouse Cytokines and Chemokines," *American Journal of Reproductive Immunology* 75, no. 6 (2016): 678–693, https://doi.org/10.1111/aji.12512.

28. A. A. Freedman, L. S. Keenan-Devlin, A. Borders, G. E. Miller, and L. M. Ernst, "Formulating a Meaningful and Comprehensive Placental Phenotypic Classification," *Pediatric and Developmental Pathology* 24, no. 4 (2021): 337–350, https://doi.org/10.1177/10935266211008444.

29. T. Y. Khong, E. E. Mooney, I. Ariel, et al., "Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement," *Archives of Pathology & Laboratory Medicine* 2016;140(7):698–713, https://doi.org/10.5858/arpa.2015-0225-CC.

30. A. Dobin, C. A. Davis, F. Schlesinger, et al., "STAR: Ultrafast Universal RNA-Seq Aligner," *Bioinformatics* 29, no. 1 (2013): 15–21, https://doi.org/10.1093/bioinformatics/bts635.

31. T. R. Fenton and J. H. Kim, "A Systematic Review and Meta-Analysis to Revise the Fenton Growth Chart for Preterm Infants," *BMC Pediatrics [Electronic Resource]* 13 (2013): 59, https://doi.org/10.1186/1471-2431-13-59.

32. S. W. Cole, W. Yan, Z. Galic, J. Arevalo, and J. A. Zack, "Expression-Based Monitoring of Transcription Factor Activity: The TELiS Database," *Bioinformatics* 21, no. 6 (2005): 803–810, https://doi.org/10.1093/bioinformatics/bti038.

33. D. M. Mosser and X. Zhang, "Interleukin-10: New Perspectives on an Old Cytokine," *Immunological Reviews* 226, no. 1 (2008): 205–218, https://doi.org/10.1111/j.1600-065X.2008.00706.x.

34. S. B. Cheng and S. Sharma, "Interleukin-10: A Pleiotropic Regulator in Pregnancy," *American Journal of Reproductive Immunology* 73, no. 6 (2015): 487–500, https://doi.org/10.1111/aji.12329.

35. V. Carlini, D. M. Noonan, E. Abdalalem, et al., "The Multifaceted Nature of IL-10: Regulation, Role in Immunological Homeostasis and Its Relevance to Cancer, COVID-19 and Post-COVID Conditions," *Frontiers in Immunology* 14 (2023): 1161067, https://doi.org/10.3389/fimmu.2023.1161067.

36. S. Kalkunte, R. Boij, W. Norris, et al., "Sera From Preeclampsia Patients Elicit Symptoms of human Disease in Mice and Provide a Basis for an In Vitro Predictive Assay," *American Journal of Pathology* 177, no. 5 (2010): 2387–2398, https://doi.org/10.2353/ajpath.2010.100475.

37. S. P. Murphy, N. N. Hanna, L. D. Fast, et al., "Evidence for Participation of Uterine Natural Killer Cells in the Mechanisms Responsible for Spontaneous Preterm Labor and Delivery," *American Journal of Obstetrics and Gynecology* 200, no. 3 (2009): 308.e1–9, https://doi.org/10.1016/j.ajog.2008.10.043.

38. S. P. Murphy, L. D. Fast, N. N. Hanna, and S. Sharma, "Uterine NK Cells Mediate Inflammation-Induced Fetal Demise in IL-10-Null Mice," *Journal of Immunology* 175, no. 6 (2005): 4084–4090, https://doi.org/10.4049/jimmunol.175.6.4084.

39. S. A. Robertson, A. S. Care, and R. J. Skinner, "Interleukin 10 Regulates Inflammatory Cytokine Synthesis to Protect Against Lipopolysaccharide-Induced Abortion and Fetal Growth Restriction in Mice," *Biology of Reproduction* 76, no. 5 (2007): 738–748, https://doi.org/10.1095/biolreprod.106.056143.

40. S. Sharma, "Natural Killer Cells and Regulatory T Cells in Early Pregnancy Loss," *International Journal of Developmental Biology* 58, no. 2 (2014): 219–229, https://doi.org/10.1387/ijdb.140109ss.

41. P. J. Katzman, "Chronic Inflammatory Lesions of the Placenta," *Seminars in Perinatology* 39, no. 1 (2015): 20–26, https://doi.org/10.1053/j.semperi.2014.10.004.

42. P. J. Katzman, S. P. Murphy, and D. A. Oble, "Immunohistochemical Analysis Reveals an Influx of Regulatory T Cells and Focal Trophoblastic STAT-1 Phosphorylation in Chronic Villitis of Unknown Etiology," *Pediatric and Developmental Pathology* 14, no. 4 (2011): 284–293, https://doi.org/10.2350/10-09-0910-OA.1.

43. P. J. Katzman and D. A. Oble, "Eosinophilic/T-Cell Chorionic Vasculitis and Chronic Villitis Involve Regulatory T Cells and Often Occur Together," *Pediatric and Developmental Pathology* 16, no. 4 (2013): 278–291, https://doi.org/10.2350/12-10-1258-OA.1.

44. R. P. Donnelly, H. Dickensheets, and D. S. Finbloom, "The Interleukin-10 Signal Transduction Pathway and Regulation of Gene Expression in Mononuclear Phagocytes," *Journal of Interferon & Cytokine Research* 19, no. 6 (1999): 563–573, https://doi.org/10.1089/107999099313695.

45. J. E. Thaxton and S. Sharma, "Interleukin-10: A Multi-Faceted Agent of Pregnancy," *American Journal of Reproductive Immunology* 63, no. 6 (2010): 482–491, https://doi.org/10.1111/j.1600-0897.2010.00810.x.

46. M. C. Nath, H. Cubro, D. J. McCormick, N. M. Milic, and V. D. Garovic, "Preeclamptic Women Have Decreased Circulating IL-10 (Interleukin-10) Values at the Time of Preeclampsia Diagnosis: Systematic Review and Meta-Analysis," *Hypertension* 76, no. 6 (2020): 1817–1827, https://doi.org/10.1161/HYPERTENSIONAHA.120.15870.

47. R. Romero, Y. M. Kim, P. Pacora, et al., "The Frequency and Type of Placental Histologic Lesions in Term Pregnancies With Normal Outcome," *Journal of Perinatal Medicine* 46, no. 6 (2018): 613–630, https://doi.org/10.1515/jpm-2018-0055.

48. L. M. Ernst, E. Basic, A. A. Freedman, E. Price, and S. Suresh, "Comparison of Placental Pathology Reports from Spontaneous Preterm Births Finalized by General Surgical Pathologists versus Perinatal Pathologist: A Call to Action," *American Journal of Surgical Pathology* 47, no. 10 (2023): 1116–1121, https://doi.org/10.1097/PAS.0000000000002111.

49. O. Shynlova, L. Nadeem, J. Zhang, C. Dunk, and S. Lye, "Myometrial Activation: Novel Concepts Underlying Labor," *Placenta* 92 (2020): 28–36, https://doi.org/10.1016/j.placenta.2020.02.005.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.