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Prospective association of maternal immune proinflammatory responsivity and regulation in pregnancy with length of gestation

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Abstract

Problem: The immune system represents a leading pathway of interest in the pathophysiology of preterm birth. The majority of human clinical studies interrogating this pathway have utilized circulating immune biomarkers; however, these concentrations typically reflect only basal production but not key functional properties of the immune system, particularly variation in the pro-inflammatory response to antigen challenge and the regulation of this response. Thus, in this study, we utilized an *ex vivo* stimulation protocol that quantifies these processes, and we examined their prospective association with the gestation length and risk of preterm birth.

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Method of Study: Immune responsiveness and regulation were assessed in 128 pregnant women in mid gestation using an *ex vivo* stimulation protocol. Maternal pro-inflammatory responsivity of leukocytes was quantified by assessing the release of the proinflammatory cytokines IL-6, TNF-α, and IL-1β in response to antigen stimulation, and regulation of the pro-inflammatory response was quantified by assessing the suppression of stimulated cytokine response upon co-incubation with increasing dexamethasone concentrations (i.e., glucocorticoid receptor resistance; GRR).

Results: Higher maternal GRR, indicating impaired regulation of the proinflammatory response, was significantly and independently associated with shorter gestational length (β= −0.42, p=0.0091) and a 3.0-fold increase in risk for preterm birth (OR= 3.01, 95%CI= 1.17-7.70, p=0.0218). Basal circulating IL-6 and TNF-α were not associated with either outcome.

Conclusions: The association of maternal GRR with length of gestation and preterm birth risk suggests that the processes represented by this measure -- maternal pro-inflammatory propensity and immune regulation -- may provide further mechanistic insight into the pathophysiology of preterm birth.

Keywords

cortisol; glucocorticoid resistance; inflammation; interleukin-1 beta; interleukin-6; length of gestation; preterm birth; tumor necrosis factor

1. Introduction:

Preterm birth constitutes one of the most important global public health challenges in maternal and child health.[1,2] A substantial body of research over the past three decades has suggested that maternal stress-related pathophysiological processes during pregnancy represent significant and independent risk factors for shorter length of gestation and preterm birth.[3] In this context, the immune/inflammatory pathway represents one of the primary biological processes, with immune alterations leading to inflammation and its downstream sequelae on maternal, placental and fetal tissues involved in parturition.[4] However, studies that have examined the role of maternal systemic immune/inflammatory biomarkers in this context have yielded inconsistent findings.[5-9] We suggest that one reason for this inconsistency may relate to the manner in which the maternal immune system has been interrogated. The majority of previous studies have utilized circulating levels of immune/inflammatory biomarkers – primarily proinflammatory cytokines in maternal circulation – to characterize the inflammatory state. While circulating concentrations of cytokines reflect basal production, they may not capture other relevant key properties of the immune system, particularly variation in the proinflammatory response to antigen challenge, and the regulation of this response. One of the primary regulators of the inflammatory response is cortisol, the end product of the hypothalamic-pituitary-adrenal axis, which plays a key and well-recognized role in down-regulating and dampening inflammation.[10-12]

Based on the consideration that in the context of pregnancy an immune phenotype characterized by greater responsivity to antigen challenge and/or reduced sensitivity to the immunosuppressive effect of cortisol may confer increased risk for adverse outcomes, we suggest that measures that capture these key properties of the maternal immune system

may prove useful over and beyond measures of circulating basal concentrations of immune biomarkers. Thus, in this study, we utilized measures of the maternal immune system that captures variation in both *a*) the inflammatory responsivity of immune cells to antigen challenge, and *b*) the magnitude of suppression of the inflammatory response by cortisol. The protocol to obtain these measures involves the collection and *ex vivo* stimulation of maternal blood with an ecologically-relevant antigen challenge (lipopolysaccharide (LPS), a bacterial cell wall endotoxin), and co-incubation with cortisol. Because the action of cortisol on immune cells is mediated by the glucocorticoid receptor (GR), this protocol has been used to characterize GR resistance in these cells (GRR). [13]

The goal of the present study was to investigate whether these measures of the functional integrity of the maternal immune system were prospectively associated with the length of gestation and the likelihood of preterm birth. We conducted the study protocol in mid-gestation in order to ensure that our measures captured these immune phenotypes at a point in time that preceded and was not a result of the labor process. We hypothesized that pregnant women whose immune cells exhibited higher inflammatory responsivity to antigen challenge and lower responsivity to the anti-inflammatory (regulatory) action of cortisol (*i.e.*, higher GR resistance) would be more likely to deliver earlier than women whose immune cells did not exhibit these features.

2. Methods

2.1 Participants

The study population was comprised of 158 women attending prenatal care at the Magee Women's Hospital of the University of Pittsburgh in Pittsburgh, PA., who were enrolled in a larger multi-site prospective cohort study (Measurement of Maternal Stress study (MOMS)). Women were eligible for the MOMS study if they were English speaking, >18 years age, with a singleton, intrauterine pregnancy, and less than 20 weeks pregnant. Exclusion criteria included major fetal congenital or chromosomal anomalies, or corticosteroid or progesterone treatment. Women at the Magee study site underwent additional study procedures, including the leukocyte *ex vivo* stimulation protocol. Once enrolled, participants attended a mid-gestation study visit, between 12 and 20 wks gestation (16.6± 2.4 wks (mean± SD)). The Institutional Review Board of the University of Pittsburgh approved the protocol. Of the 158 women, 1 woman with an intra-uterine fetal demise was excluded from analysis. As length of gestation was the primary outcome of interest, we further excluded 12 women with elective C-sections, and 17 women with incomplete birth record data. Hence, analyses were conducted in the subset of 128 women. This subsample was representative of the sociodemographic characteristics of the larger sample (maternal age, education, race/ ethnicity $p>0.1$).

2.2 Sociodemographic and Clinical Data

Standardized structured interviews were conducted for ascertainment of maternal sociodemographic characteristics (maternal age, education, race/ ethnicity). Women self-reported their race/ethnicity from the following categories: a) white, b) Black, c) Hispanic, d) Other. No women self-reported as Hispanic. Because Black women are at higher

risk of pre-term birth the race/ethnicity variable was coded as Black versus non-Black (non-Hispanic white + other). Maternal socioeconomic status (SES) was defined as a combination of maternal educational level (assessed in categories from less than high school to advanced degree (master/doctorate) and coded into values from 1 to 3) and household income (originally assessed in categories from ≤ $15,000 to ≥ $100,000 and then coded into values from 1 to 4). The individual education level and household income were standardized (mean=0 and SD=1) and then summed for the combined SES index. Obstetric risk was classified as being "present" if either of the following were present: pre- or gestational diabetes or chronic hypertension. Additionally, presence of peri-partum infection was abstracted from the medical record and included clinical evidence of chorioamnionitis, pyelonephritis or pneumonia, and was included as another risk factor. Parity was coded as a 0 (no previous pregnancy), 1 (one previous pregnancy), or 2 (two or more previous pregnancies). Pre-pregnancy body-mass index (BMI; weight kg/ height m^2) was computed based on pre-pregnancy weight and height. Length of gestation was abstracted from the medical record, and preterm birth was categorized as length of gestation <37 completed wks gestation.

2.3 Fasting Blood Draw and Circulating Cytokines

A fasting maternal blood draw was collected at the mid-gestation study visit via antecubital venipuncture into 10-mL EDTA-coated Vacutainer tubes (BD Biosciences), which was spun within 30 minutes of collection at 2,000 x g at 4°C for 15 minutes. Plasma was harvested and stored at −80C until the assay for circulating IL-6 and TNF-α was performed. A MSD V-Plex assay was constructed that measured the circulating IL-6 and TNF-α in duplicate,[14] and yielded reliable values (IL-6 intra-assay CV=5.54, inter-assay <10, lower limit of detection (LLD)=0.06 pg/mL; TNF-α CV=5.00, inter-assay <10, lower limit of detection (LLD)=0.04 pg/mL).

2.4 Leukocyte *Ex Vivo* Stimulation.

Immune responsiveness and regulation were assessed using an *ex vivo* stimulation protocol, based on previously published procedures.[15] In brief, venous blood was collected in heparinized sterile tubes and diluted 10:1 with sterile phosphate buffered saline. 400μL aliquots of this mixture were dispensed into wells containing a) lipopolysaccharide (LPS from Escherichia coli, 055:B5, Sigma-Aldrich; 60ng/mL) alone, and b) LPS (60ng/mL) co-incubated with serial dilutions of dexamethasone (Sigma-Aldrich; final concentration 10^{-7}, 10^{-6}, and 10^{-5}M), on a 24-well plate. After 6-h of incubation at 37 °C and 5% CO2, the plates were centrifuged at 2000 *x* g at 4°C for 10 min and the plasma supernatant was collected and stored at −80°C until analysis. Assays were conducted in duplicate of the pro-inflammatory cytokine concentrations, IL-6, TNF-α, IL-1β, and INF- γ, using a commercially available Multiplex Bead-Based Kit, the V-PLEX Proinflammatory Panel 1 (4-Plex, Human Panel; Millipore, Billerica, MA, USA) in accordance with the kit-specific protocols. Plates were read on a Luminex FLEXMAP 3D System and analyzed using xPONENT® software (Luminex). Values below the detection limit were set to the plate-specific lowest standard concentration (IL-6 2.4%, TNF-α 0%, IL-1β 1.0%, and INF-γ 2.0% of sample). Three cytokines, IL-6, TNF-α, and IL-1β, yielded reliable values (intra-assay CV=6.02, LLD=0.06 pg/mL; CV=5.42, LLD= 0.04 pg/mL; CV=6.49, LLD=

0.04 pg/mL; respectively) and were used in the following calculations (INF- γ was excluded since the intra-assay CV>10). The release by leukocytes of the pro-inflammatory cytokines after co-incubation with LPS (alone) was measured and provided a marker of pro-inflammatory responsiveness to a standardized antigen challenge (referred to as stimulated cytokine). Furthermore, the ability of cortisol (represented by standard dexamethasone) to suppress or dampen stimulated cytokine release across the four cortisol gradient exposures (LPS+0, LPS+10^{-7}, LPS+10^{-6}, and LPS+10^{-5}M dexamethasone) was quantified using standard linear trapezoidal area under the curve (AUC) calculation,[16] and provides a marker of the extent to which cortisol regulates the inflammatory response (i.e., glucocorticoid receptor resistance (GRR)), with higher cytokine AUC indicating greater GRR. A composite pro-inflammatory cytokine score was calculated from the three available cytokines (IL-6, TNF-α, and IL-1β), by standardizing (z-score) the stimulated cytokine release, and the GRR AUC for each cytokine, to ensure even weighting, and then calculating a standardized mean for both values.

2.5 Statistical Methods

Descriptive statistics were used to describe baseline maternal sociodemographic characteristics. Circulating plasma, and *ex vivo* stimulated cytokine concentrations were natural log-transformed, furthermore, the Ln transformed values of the cytokine concentrations from the ex vivo stimulation studies were standardized before computing the mean stimulated cytokine score and GRR, and all modeled betas, confidence intervals and p-values and model interpretations represent the Ln and Ln z-scored transformed values; however, the descriptive tables are presented in the untransformed form for ease of interpretation of the values.

The associations between circulating cytokines, pro-inflammatory responsivity score, and regulation of inflammation (GRR) score were assessed by bivariate correlation. Next, the association between these three predictors with length of gestation were examined using linear regression analysis, and with preterm birth (<37 weeks) using logistic regression. Taking into account of possibility of heteroscedasticity (i.e. non-consistent modeling error), the robust standard error estimates of the regression coefficient estimates were obtained in order to make unbiased inferences of hypothesis tests in the regression coefficients.[17] Because the effects of several maternal factors during pregnancy have been shown to vary as a function of the sex of the fetus,[3] our analyses included an interaction term with fetal sex in the multivariable models (non-significant interaction terms are not included in final models).

The following *a priori* selected covariates were included in all adjusted models: maternal age, parity, race/ethnicity, SES, pre-pregnancy BMI, gestational age at assessment, peri-partum infection, fetal sex, and OB risk. Although maternal BMI can have a non-linear association with obstetric outcomes,[18] there were no underweight (<18.5 kg/m^2) women in this sample, and therefore BMI was used as a continuous variable. Because basal and stimulated cytokine concentrations can vary as a function of gestational age, all models were adjusted accordingly to account for gestational age at assessment.[19] All statistical analyses were performed with SAS® Software Version 9.4. Results were considered statistically significant at the level of $p < 0.05$.

3. Results

The descriptive statistics of maternal sociodemographic characteristics, predictors, covariates, and outcomes are reported in Table 1. Women had a mean (± standard deviation) age of 30.2±5.3 years and a pre-pregnancy BMI of 28.2±7.8 kg/m^2. 7.8% of births were pre-term (10/128), of which 9 were spontaneous and 1 was medically indicated. The reported results include the medically indicated induction, but exclusion of this case did not change any findings.

We examined the association among the maternal circulating pro-inflammatory cytokine concentrations (circulating plasma IL-6 and TNF-α), the LPS-stimulated proinflammatory cytokine responsivity scores, and the regulation of inflammatory responses by cortisol (GRR: cytokine score AUC). There was no significant association between circulating plasma IL-6 concentrations and either *ex vivo* stimulation measure (LPS-stimulated cytokine score: r= 0.12, p>0.05; GRR: r=−0.02, p>0.05). Similarly, there was no association between circulating plasma TNF-α and the stimulated cytokine score (r= −0.09, p>0.05), but there was a significant and positive association with the GRR cytokine score (r= 0.26, p= 0.0027). As expected, there was a significant and positive association between LPS-stimulated cytokine release and the GRR cytokine suppression score (r=0.54, p<0.001).

3.1 Maternal pro-inflammatory profile and gestational length

The circulating concentrations of IL-6 and TNF-α in mid-gestation were not significantly associated with gestational length (IL-6: β= −0.36, p=0.279; adjusted β= 0.11, p=0.771; TNF-α: β= −0.08, p=0.919; adjusted β= 0.99, p=0.10) or pre-term birth (IL-6: Ln-unit OR= 1.46, 95% CI = 0.48-4.42, p=0.503; Ln-unit adjusted OR= 0.49, 95%CI=0.05-5.28 , p=0.559; TNF-α: Ln-unit OR= 2.52, 95% CI = 0.19-34.07, p=0.486; Ln-unit adjusted OR= 0.45, 95% CI=0.02-9.76 , p=0.613).

Maternal pro-inflammatory responsivity (LPS-stimulated cytokine score) was not associated with gestational length (β= −0.24, p=0.124, Figure 1a ;Table 2a), however, after inclusion of covariates there was a marginal association, such that greater proinflammatory responsivity (higher stimulated cytokine concentration) was associated with shorter length of gestation (adjusted β= −0.32, p=0.0785). Stimulated cytokine concentration was not significantly associated with odds of pre-term birth (Ln-unit OR= 1.54, 95% CI= 0.65-3.66, p=0.322; Ln-unit adjusted OR= 2.29, 95%CI= 0.53-9.85, p=0.265; Figure 1b).

As shown in Figure 1, impaired regulation of the inflammatory response (higher GRR) was significantly associated with shorter gestational length (β= −0.55, p=0.0084; Figure 1c, Table 2b). This association remained significant after accounting for the sociodemographic and obstetric covariates (adjusted β= −0.42, p=0.009). Moreover, regulation of inflammatory responsivity (i.e., GRR) also was significantly associated with increased odds of preterm birth (Ln-unit OR= 2.00, 95% CI= 1.12-3.52, p=0.019; Ln-unit adjusted OR= 3.01, 95%CI= 1.17-7.70, p=0.0218; Figure 1d and Figure 2). Figure 2 depicts the mean pro-inflammatory cytokine release across the 4 conditions of LPS stimulation and increasing cortisol (represented by standard dexamethasone) concentrations and demonstrates the impaired

regulation of the inflammatory cytokine release in women who deliver preterm compared to women who deliver term.

Finally, when circulating IL-6, TNF-α, the LPS-stimulated composite proinflammatory cytokine measure, and the GRR measure were included in the same model, GRR remained the only significant predictor of length of gestation, before and after controlling for co-variates (adjusted β= −0.64, p=0.0029). All associations with length of gestation and preterm birth were consistent across fetal sex (p>0.1 for interaction term).

4. Discussion

Consistent with our hypotheses, the findings of this study suggest that while circulating biomarkers of maternal inflammation were not prospectively associated with length of gestation, measures of maternal immune responsivity to antigen stimulation and of regulation of inflammatory response by cortisol were prospectively associated with length of gestation. In particular, the measure quantifying individual differences in the regulation of the maternal inflammatory response to antigen (i.e., maternal immune cell glucocorticoid resistance (GRR)), remained significant after adjustment for sociodemographic and obstetric covariates. A 1 standard deviation difference in GRR was independently associated with a 1.1 week decrease in gestational age at birth and a 3-fold higher risk for preterm birth. To the best of our knowledge, these findings represent the first report showing impaired dampening by cortisol of the pro-inflammatory cytokine response to antigen is prospectively associated with length of gestation and risk of preterm birth. The finding provides mechanistic insight into cellular immune regulation that cannot be gleaned from the circulating inflammatory biomarkers used in most previous studies.

Previous reports of the relationship between circulating maternal biomarkers have been inconsistently associated with ~1.2-1.5 greater risk for preterm birth[6,7,9,20], whereas the findings of this study suggest a novel maternal phenotype of inflammatory propensity is associated with a 3-fold increase preterm birth risk. Furthermore, because our study population was comprised primarily of healthy (low obstetric risk) women, our findings likely represent a conservative estimate of the effect size in a high-risk population.

The role of inflammatory processes in the initiation and progression of parturition is well established.[6,21] However excess inflammation in pregnancy is associated with poor maternal and birth outcomes, and the most strongly predictive markers are from matrices close to the maternal-fetal interface, such as amniotic and cervicovaginal fluid.[9] Markers measured in maternal circulation have proven less useful, in part because of their decreased proximity to the gestational tissues that undergo change with advancing gestation, but in addition, circulating concentrations typically reflect low levels of basal production, and do not capture other key properties of the immune system, particularly variation in the proinflammatory response to antigen challenge and the regulation of this response. Our study data support the importance of quantifying this maternal phenotype of inflammatory propensity beyond markers found in circulation. Indeed, there was no prospective association between either circulating cytokine, IL-6 or TNF-α, and length of gestation, but rather, an association of gestational length with the dampening of stimulated cytokine release in response to

challenge by cortisol. Notably, in our data, we measured the dampening of stimulated cytokine release by cortisol across a comprehensive panel of three pro-inflammatory cytokines, IL-6, TNF-α, and IL-1β, and although we present the association with the composite mean cytokine score, the finding was consistent across all three cytokines (data not shown). Importantly, this association was evident in immune cells collected from circulation and may represent a systemic inflammatory propensity of the maternal immune system, however it would be beneficial in future studies to measure the GRR response in biological fluid or other relevant tissues closer to the maternal-fetal interface.

Under normal conditions, cortisol is potently anti-inflammatory, and stimulation of the HPA axis by stressful stimuli or pro-inflammatory cytokines, results in cortisol release and dampening of systemic inflammation. Glucocorticoid receptors (GR) are located in the cytoplasm, and once bound by cortisol, GR translocates to the nucleus, and inhibits nuclear factor-κB (NF-κB) production and suppresses the phosphorylation of mitogen-activated protein kinase (MAPK), which dampens the production of pro-inflammatory cytokines, such as the IL-6, TNF-α, and IL-1β measured in this study.[22,23] However, in the face of chronic stress, immune cells can become less sensitive to cortisol[10,12,24], as measured by GRR, suppressing this essential feedback loop. It has previously been shown that GRR naturally rises across gestation,[25] however women with the highest resistance to cortisol, particularly mid-gestation as measured in this study, may indicate a susceptible maternal system. As evident from Figure 2, the differences in pro-inflammatory cytokine concentrations between mothers who delivered at term and those who delivered preterm were relatively modest upon LPS stimulation and after co-incubating with a relatively high concentration of cortisol, but differences were particularly pronounced in the lower or moderate concentrations of cortisol. This aptly illustrates the substantial impairment in the ability of cortisol (at moderate or physiological levels) to regulate the inflammatory response in women who eventually deliver preterm. GRR was the only significant *independent* predictor of gestational length, even when all inflammatory measures were included in the same statistical model. This highlights the importance of quantification of the pro-inflammatory immune regulation above and beyond the inflammatory immune ligands as a prospective maternal phenotype. Furthermore, cortisol bound GR not only interacts with nuclear pro-inflammatory transcription pathways as described above, but it also exerts other nuclear and extranuclear actions, and therefore increased GRR may lead to other cellular changes beyond impaired immune regulation that could mediate the risk for preterm birth. We specifically measured GR actions tied to inflammatory signaling, but other GR cellular targets should be considered in future studies.

Study limitations include the modest sample size and number of preterm births. Future studies with larger sample size and greater diversity in race/ethnicity should utilize these *ex vivo* stimulation measures of glucocorticoid and immune function, as this could be particularly informative for racial disparities research, given that women of racial and ethnic monitories are at increased risk for PTB. A study using a surrogate marker for glucocorticoid resistance showed minority women also exhibited elevated GRR[26]; therefore, we suggest GRR could be investigated as a potential mediator for racial disparities in preterm birth.[27] Because study inclusion was limited to relatively healthy women and PTB cases were primarily spontaneous in etiology, future studies should also investigate the role

of GRR in populations enriched for medically-indicated preterm births. Furthermore, even though our models adjusted for presence of infection at the time of delivery, this study is limited by lack of measurement of subclinical infection at the time of blood sampling.

In summary, maternal immune/inflammatory regulation of pro-inflammatory cytokine release is associated with shorter length of gestation and increased risk for pre-term birth. These *ex vivo* stimulation measures quantify a novel maternal phenotype of proinflammatory propensity and could be included in future studies, Our findings also support the importance of considering variation in systems that regulate inflammatory signaling and provide novel mechanistic data in informing the underlying pathophysiology of preterm birth.

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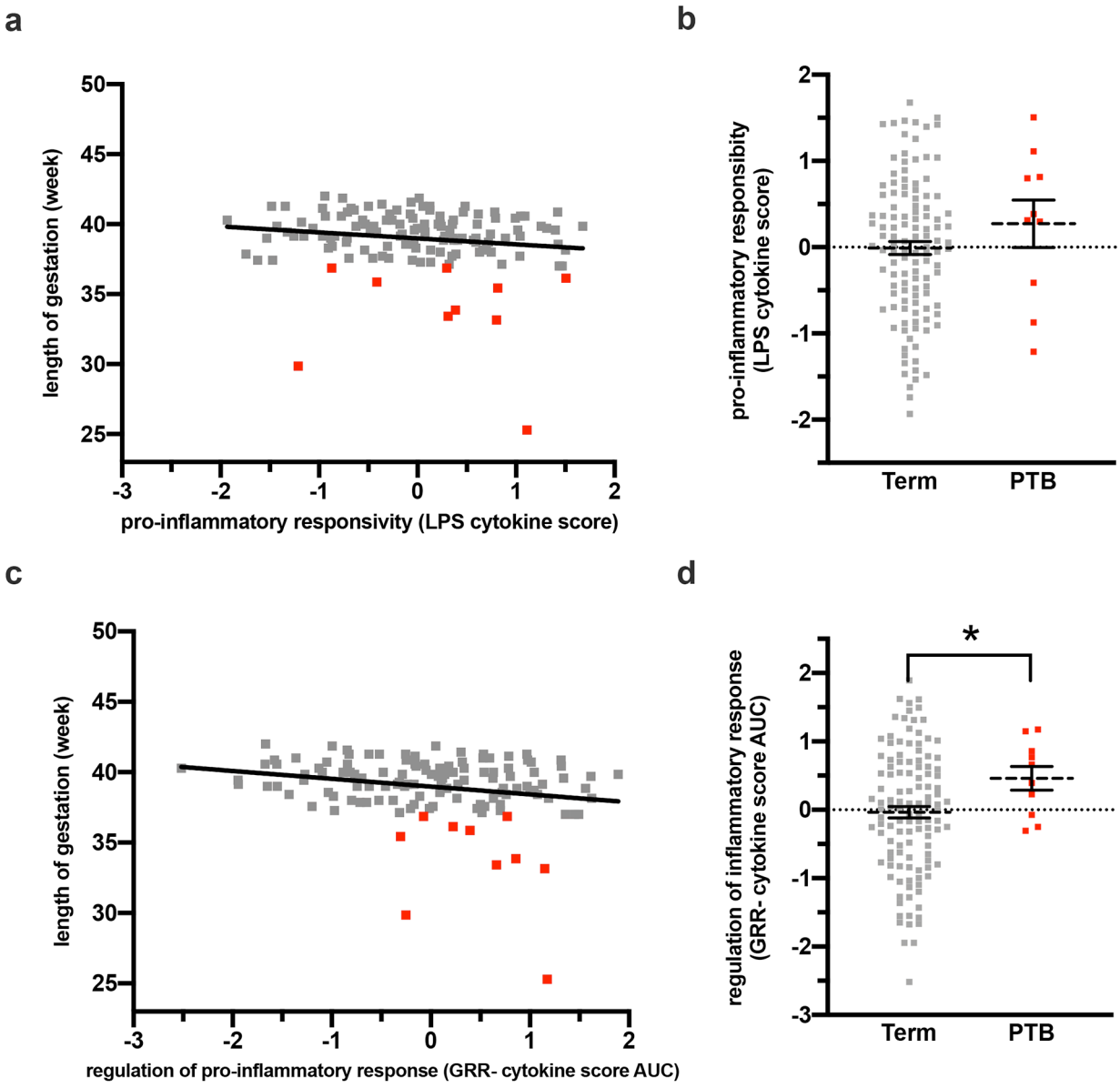

Figure 1. Pro-inflammatory cytokine regulation is associated with gestational length and preterm birth risk.

LPS-*stimulated cytokine responsivity* is associated with **a)** length of gestation, but only after adjustment for sociodemographic and obstetric covariates (β= −0.24, p=0.124; adjusted β= −0.32, p=0.0785), and **b)** is not associated with risk for preterm birth before or after covariate adjustment (Ln-unit OR= 1.54, 95% CI= 0.65-3.66, p=0.322; Ln-unit adjusted OR= 2.29, 95%CI= 0.53-9.85, p=0.265).

The *regulation of cytokine responsivity (cytokine score AUC, representing glucocorticoid receptor resistance (GRR))* is significantly associated with **c)** length of gestation (β= −0.55, p=0.0084; adjusted β= −0.42, p=0.009), and **d)** risk for pre-term birth - before and after adjustment for sociodemographic and obstetric covariates (Ln-unit OR= 2.00, 95% CI= 1.12-3.52, p=0.019; Ln-unit adjusted OR= 3.01, 95%CI= 1.17-7.70, p=0.0218).

Across all figures, data points for women who delivered pre-term are indicated by the color **red**.

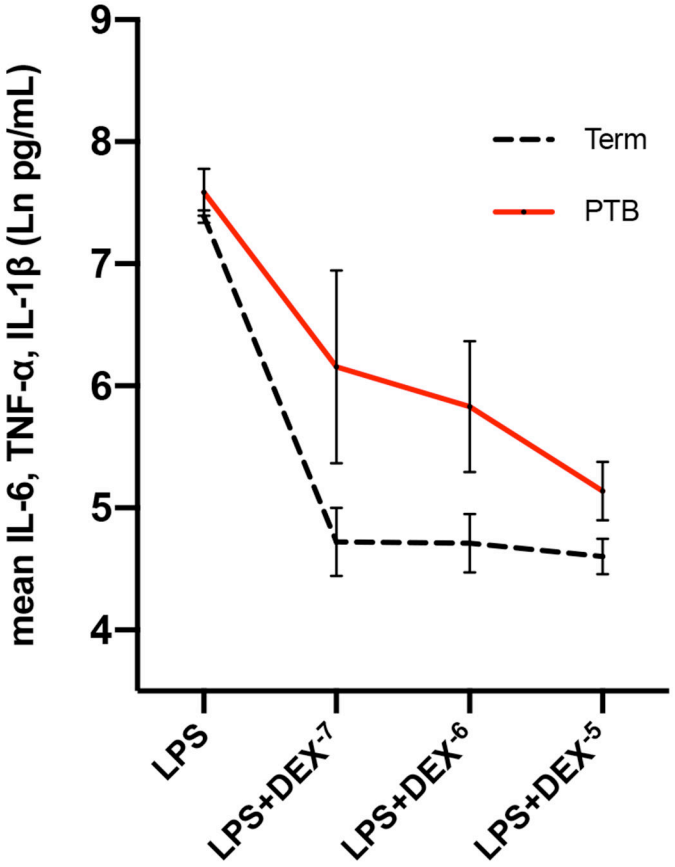

Figure 2. ***Ex vivo*** **stimulation protocol: pro-inflammatory cytokine regulation and risk for preterm birth**

This figure depicts the data from the *ex vivo* stimulation protocol, showing the mean ± standard error of the average cytokine concentrations (IL-6, TNF-α, and IL-1β) across the four lipopolysaccharide (LPS)-stimulated and cortisol dexamethasone (DEX) gradient conditions: *i*) LPS+ 0; *ii*) LPS+ 10^{-7}M; *iii*) LPS+10^{-6} M; and *iv*) LPS+10^{-5}M. These data suggest that among women who delivered preterm (PTB) the ability of cortisol to downregulate the LPS-stimulated cytokine production is reduced (*i.e.*, greater glucocorticoid receptor resistance; GRR) compared to women who delivered at term.

Table 1-

Descriptive statistics of key maternal predictors, covariates and study outcomes

Variable	Mean ± SD	Q1, Q3 (IQR)
Maternal predictors:		
Circulating plasma IL-6 (pg/mL)	0.62±0.53	0.36, 0.70 (0.33)
Circulating plasma TNF-α (pg/mL)	0.94±0.33	0.72, 1.10 (0.38)
LPS-Stimulated IL-6 (pg/mL)	3,486±2,240	1835, 4487 (2652)
LPS-Stimulated TNF-α (pg/mL)	888±513	510, 1120 (609)
LPS-Stimulated IL-1β (pg/mL)	2,676±2,085	1178, 3409 (2231)
LPS-Stimulated Composite Cytokine Score	0.013±0.821	−0.59, 0.60 (1.19)
GRR: IL-6 AUC (pg/mL)	4,495±3,561	1972, 5830 (3858)
GRR: TNF-α AUC (pg/mL)	1,331±1,072	510, 1761 (1761)
GRR: IL-1β AUC (pg/mL)	3,512±3,169	1504, 4908 (3403)
GRR: Composite Cytokine Score AUC	0.003±0.900	−0.64, 0.70 (1.34)
Maternal covariates:		
Age (years)	30.2±5.3	26.7, 33.4 (6.7)
SES index	0.04±1.82	−1.17, 1.18 (2.35)
Pre-pregnancy BMI (kg/m^2)	28.2±7.8	22.8, 32.4 (9.6)
Gestational age at assessment (weeks)	16.7±2.4	14.7, 18.7 (4.0)
BMI categories:		
Normal weight	62(48.4%)	-
Overweight	24(18.8%)	-
Obese	42(32.8%)	-
Race/ethnicity:		
Black	25(19.5%)	-
Non-Hispanic White	99(77.3%)	-
Other	4(3.1%)	-
Obstetric risk pregnancy (%yes)	16(12.5%)	-
Parity:		
0	55(43.0%)	-
1	47(36.7%)	-
≥2	26(20.3%)	-
Peri-partum infection	4(3.1%)	-
Child and birth outcomes:		
Gestational length (weeks)	39.0±2.2	38.1, 40.3 (2.1)
Preterm birth (% yes)	10 (7.8%)	-
Child sex (%male)	71 (55.5%)	-

Continuous variables are described using mean± standard deviation (SD), with the 1st and 3rd quartile (Q1, Q3) and interquartile range (IQR). Categorical variables are described using frequency(percent%).

Abbreviations: IL-6= interleukin-6, TNF-α= tumor necrosis factor-α, IL-1β= interleukin-1β, LPS= lipopolysaccharide, GRR= glucocorticoid receptor resistance, AUC= area under the curve, SES= socioeconomic status, BMI= body mass

Table 2a-

Prospective association between pro-inflammatory cytokine responsivity (LPS-stimulated composite cytokine score) and length of gestation

	Ind. Variable	*β*	SE	95% CI		p
model 1 - unadjusted	**stimulated cytokine**	−0.43	0.27	−0.96	0.108	0.1175
model 2- adjusted with *a priori* covariates	**stimulated cytokine**	−0.32	0.18	−0.68	0.04	0.0785
	race/ethnicity *	−0.56	0.51	−1.57	0.44	0.2738
	pre-pregnancy BMI	−0.07	0.05	−0.16	0.03	0.1588
	age	0.05	0.05	−0.04	0.15	0.2808
	infant sex *	−0.44	0.32	−1.06	0.19	0.1701
	parity 1 *	−0.29	0.39	−1.05	0.48	0.4607
	parity 2+ *	0.19	0.55	−0.89	1.27	0.7339
	OBrisk *	−1.02	0.5	−2	−0.04	**0.0414**
	SES score	−0.02	0.11	−0.24	0.19	0.8482
	gestational age at assessment	0.18	0.09	0.02	0.35	**0.0324**
	peri-partum infection *	−0.12	0.79	−1.67	1.42	0.8756

* race/ethnicity is coded as Black vs non-Black (non-Hispanic white + other) with white/other as the reference group

* female is the reference group for infant sex

* nulliparous is the reference group for parity 1 and parity 2+

* "no" OBrisk is the reference group for OBrisk

* "no" peri-partum infection is the reference group for peri-partum infection; **Bold** text indicates when $p < .05$.

Table 2b-

Prospective association between the regulation of pro-inflammatory response (GRR- composite cytokine score AUC) and length of gestation

	Ind. Variable	*β*	SE	95% CI		p
model 1 - unadjusted	**GRR**	−0.55	0.21	−0.97	−0.14	**0.0084**
model 2- adjusted with *a priori* covariates	**GRR**	−0.42	0.16	−0.73	−0.1	**0.0091**
	race/ethnicity *	−0.58	0.52	−1.59	0.43	0.2567
	pre-pregnancy BMI	−0.07	0.05	−0.16	0.02	0.1265
	age	0.05	0.05	−0.05	0.14	0.3487
	infant sex *	−0.55	0.31	−1.16	0.07	0.0812
	parity 1 *	−0.24	0.38	−0.99	0.51	0.5258
	parity 2+ *	0.23	0.55	−0.85	1.3	0.6819
	OBrisk *	−0.99	0.48	−1.93	−0.06	**0.0367**
	SES score	0.00	0.11	−0.21	0.21	0.9923
	gestational age at assessment	0.14	0.08	−0.02	0.31	0.0936
	peri-partum infection *	−0.01	0.91	−1.79	1.76	0.9870

* race/ethnicity is coded as Black vs non-Black (non-Hispanic white + other) with white/other as the reference group

* female is the reference group for infant sex

* nulliparous is the reference group for parity 1 and parity 2+

* "no" OBrisk is the reference group for OBrisk

* "no" peri-partum infection is the reference group for peri-partum infection; **Bold** text indicates when $p < .05$.