HHS Public Access

Author manuscript

Am J Reprod Immunol. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Am J Reprod Immunol. 2020 July ; 84(1): e13252. doi:10.1111/aji.13252.

Glucocorticoid receptor sensitivity in early pregnancy in an African American cohort

Lasha S Clarke[a], **Elizabeth Corwin**[b], **Anne Dunlop**[b], **Allison Hankus**[a], **Josh Bradner**[c], **Sudeshna Paul**[b], **Yunshen Jiao**[a], **Alicia K Smith**[d], **Nikolay Patrushev**[b], **Jennifer Mulle**[c], **Timothy D Read**[e], **Carol JR Hogue**[a], **Bradley D Pearce**[a]

[a]Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd, Atlanta, Georgia, 30322, USA

[b]Nell Hodgson Woodruff School of Nursing, Emory University, 1520 Clifton Rd, Atlanta, Georgia, 30322, USA

[c]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia 30322, USA

[d]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, 101 Woodruff Cir NE, Atlanta, Georgia, 30322, USA

[e]Department of Medicine, Department of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia, 30322, USA

Abstract

Problem—Disruption in homeostatic feedback loops between inflammatory mediators and the hypothalamic-pituitary-adrenal (HPA) axis is a key mechanism linking chronic stress to inflammation and adverse health outcomes, including those occurring during pregnancy. In particular, alterations in glucocorticoid sensitivity may occur as a result of chronic stress, including that due to racial discrimination, and may be implicated in the persistent adverse maternal and infant health outcomes experienced by African Americans. While there are a few large-scale studies in human pregnancy that measure both cytokines and HPA axis hormones, none directly measured glucocorticoid sensitivity at the cellular level, especially in an African American population.

Method of study—We measured the full range of the dexamethasone (DEX) dose response suppression of TNF-α in first trimester blood samples from 408 African American women, and estimated leukocyte cell type contribution to the production of TNF-α.

Results—The mean (SD) DEX level needed to inhibit TNF-α production by 50% (i.e., DEX IC_{50}) was 9.8 (5.8) nmol/L. Monocytes appeared to be the main driver of uninhibited TNF-α production, but monocyte counts explained only 14% of the variation. Monocyte counts were only weakly correlated with the DEX IC_{50} ($r=-0.11$, $p<0.05$). Moreover, there was no statistically significant correlation between the DEX IC_{50} and circulating proinflammatory (CRP, IL-6, IFN-γ) or anti-inflammatory (IL-10) mediators ($p>0.05$).

Corresponding author: Bradley D Pearce[a], bpearce@emory.edu.

Conclusions—These findings challenge some prior assumptions and position our comprehensive study of glucocorticoid sensitivity as an important anchor point in the growing recognition of interindividual variation in maternal HPA axis regulation and inflammatory responses.

Keywords

glucocorticoid sensitivity; cytokine; dexamethasone; African American; pregnancy; C-reactive protein

INTRODUCTION

Alterations in glucocorticoid (GC) sensitivity may occur as a result of chronic stress, including that due to racial discrimination, and thus may be implicated in the persistent adverse maternal and infant health outcomes of African Americans [1]. Despite the appeal of this mechanism, there are many gaps in the literature. It is well established that African-American women experience a greater risk of several pregnancy complications—especially preterm birth (PTB)—compared to white women. Inflammation is clearly associated with PTB, and the overproduction of inflammatory mediators is a particularly prominent factor in PTB among African-Americans. For example, there is evidence that differential cytokine production patterns between term and preterm labor, and between African American and White women, explain some of the racial disparities in PTB [2, 3]. Relatedly, we have demonstrated that minority status or low income are also associated with elevated cortisol levels during pregnancy [4]. However there is substantial individual variability in both cytokine production and changes in circulating hypothalamic-pituitary-adrenal (HPA) axis hormones, which highlights the need for larger scale studies that may explain the role of neuroendocrine and immune interactions in the high rate of PTB among African-Americans.

The HPA axis is activated by physiological and psychological stress, and there is a rich literature on the interplay between stress, HPA axis activation, and the release pro- and anti-inflammatory cytokines [5, 6]. The connection between HPA axis hormones, inflammation and pregnancy complications has become a cornerstone for understanding the psychoneuroimmunology of pregnancy [7–10]. During normal gestation, the maternal HPA axis becomes progressively activated. These HPA axis hormones participate in a network with other hormones and inflammatory mediators to ultimately determine the timing of parturition. A disruption in this network is a proposed mechanism linking chronic stress to inflammation and PTB [11–17].

Compared with White women, African American women experience a greater risk of chronic stress associated with racial discrimination. In general, chronic stress is associated with increased inflammation, but there is individual variability that is likely due in part to individual differences in GC sensitivity. We and others have proposed that chronic stress due to racial discrimination causes inflammation among pregnant African-American women, but the impact of racial discrimination-associated stress on inflammation risk varies among individuals exposed to racial discrimination.

This is a complex hypothesis that requires testing by breaking it into component parts, some of which can be tested in all populations (e.g. impact of chronic stress on inflammatory processes) and some of which must be tested in specific populations (e.g. impact of racial discrimination on chronic stress and pregnancy outcome). One of those component parts is at the cellular level, testing individual variability in GC sensitivity.

To test GC sensitivity to negative feedback, studies in non-obstetrical populations have commonly measured the sensitivity of peripheral blood leukocytes to a synthetic GC that mimics cortisol [18, 19]. GC resistance is associated with loss of the normal negative association between GC concentration and indicators of inflammatory immune activation [4]. In obstetrical populations, few studies of GC sensitivity have focused on African American populations, who may experience and embody chronic exposure to racial discrimination uniquely [1, 20–23]. Moreover, there are only a few large-scale studies in human pregnancy that measured both cytokines and HPA axis hormones, and none of these directly measured glucocorticoid sensitivity at the cellular level [4, 24, 25]. Katz et al. [26] measured the expression of glucocorticoid related genes in a pregnancy sample of women with serious mental illness that included 13% African-Americans. They performed a dose-response curve for dexamethasone suppression of IL-6 in a subset of 29 women in the sample, though only two of these were collected in the first trimester [26]. Nevertheless, that study provided intriguing evidence that prenatal depressive symptoms are associated with altered regulation of GR sensitivity.

In this paper, we fill in some of the major gaps in the literature on glucocorticoid sensitivity during pregnancy. For example, while previous studies have used indirect measures of glucocorticoid sensitivity, we measured the full range of the dexamethasone (DEX) dose response in individual blood samples from over 400 women in early pregnancy. This is critical because a fundamental principle of GC sensitivity in stress and depression implies a rightward shift in this dose response curve due to insensitivity or down regulation of GR or its downstream signal transduction pathways. Our focus on early pregnancy also differentiates this study from most previous biological studies of immune variables in humans. Moreover, we test prior assumptions concerning the association of TNF production in relation to white blood cell (WBC) type. These assumptions have not been previously tested in early human pregnancy. We also examined the interrelationship between glucocorticoid sensitivity and common cytokine biomarkers used in pregnancy research. Finally, we focus on pregnant African American women and consider extensive demographic and pregnancy-related variables in our analysis.

Specifically, this study used the bacterial endotoxin lipopolysaccharide (LPS) to induce immune activation and pro-inflammatory cytokine production by leukocytes in order to: 1) describe *in vitro* immune function during early pregnancy among a large cohort of sociodemographically diverse pregnant African American women, 2) use a dose-response curve to examine inhibition of LPS-induced cytokine production by the glucocorticoid dexamethasone (DEX), in relation to serum cytokine concentrations, and 3) estimate leukocyte cell type contribution to the production of TNF-α, a multipotent inflammatory cytokine implicated in pregnancy complications [2, 27–29].

METHODS

Study design and participants

This cross-sectional analysis utilizes first trimester prenatal data collected from a subset of enrollees (n=408) in the ongoing longitudinal Emory University African American Microbiome in Pregnancy Cohort Study [30]. Study participants were women receiving prenatal care at clinics affiliated with either of two Atlanta-area hospitals: Grady Memorial Hospital, a county-supported hospital that serves as a safety net for low-income patients, and Emory University Hospital Midtown, a private hospital that serves patients from a wide economic range. Those hospitals, which together see 10% of Georgia's singleton live births to African American women, were selected to yield sufficient within-race variation in sociodemographic and biological factors of interest.

Investigators invited eligible women to participate in the study at their first prenatal visit (8–14 weeks' gestation), and followed participants through or beyond delivery. Eligible women were between 18–40 years of age, self-identified as African American (i.e., US-born and of African American or Black race), carrying a singleton pregnancy between 8–14 weeks' gestation at the time of enrollment (verified by clinical record and/or ultrasound), and experiencing no known chronic medical conditions or taking prescribed medications for chronic conditions. Some women were diagnosed with complications of pregnancy following enrollment.

The first-trimester study visit included collection of blood and completion of sociodemographic, health, nutrition and stressor exposure questionnaires. The Emory University Institutional Review Board granted approval for all data collection, and all participants provided written informed consent.

Participant demographic and health characteristics

During the first-trimester study visit, a Sociodemographic Survey was completed using participant self-report and prenatal administrative record review to gather information on maternal age, years of education, relationship status, and insurance status. Women who reported completing "at least some college" were categorized as "College educated." Those who reported being married or in a relationship, regardless of cohabitation status, were categorized as "Partnered." Insurance status was categorized as having "Public insurance," which includes participants enrolled in Medicaid, or having "Private insurance.

Enrollees completed a health survey by self-report to ascertain, within the last month, diagnoses, medication, and other health attributes. Researchers completed maternal medical chart abstraction using a standardized chart abstraction tool to ascertain parity (defined as total number of live births) and first-trimester pregnancy body mass index (BMI). First-trimester BMI was calculated from measured height and weight at the first prenatal visit, and categorized according to accepted definitions (obese: ≥30 kg/m^2, overweight: 25-<30 kg/m^2, healthy weight: 18.5-<25 kg/m^2, and underweight: <18.5 kg/m^2). All participants received early pregnancy dating by last menstrual period and/or early ultrasound, according to enrollment criteria. From record review after delivery, researchers ascertained gestational

diabetes, preeclampsia, eclampsia, etc., type and mode of delivery defined according to standard clinical definitions of the American College of Obstetricians and Gynecologists.

Biological parameters

Blood Samples—During a routine prenatal blood draw between 8–14 weeks' gestation, the laboratory technician obtained an additional 30 mL venous blood from study participants: half collected in serum tubes placed on ice prior to centrifugation then stored at −80 °C for subsequent measurement of serum cytokines and half into heparin-containing tubes that remain at room temperature for *in vitro* dexamethasone (DEX) suppression testing.

C-reactive protein—C-reactive protein (CRP) was measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN) with sensitivity to 0.022 ng/mL. The range of this assay is 0.8–5.0 ng/mL, with inter- and intra-assay variability of approximately 4% and 6%, respectively.

Cytokines—Serum samples were analyzed for cytokines, interferon (IFN)-γ, IL-6, IL-10, and TNF-α, using the MesoScale assay platform (Meso Scale Diagnostics Rockville, Maryland) according to the protocols supplied by the manufacturer. The MesoScale multiplex assay system uses electrochemiluminescence for high sensitivity and broad dynamic range. Lower limits of detection were 0.37 pg/mL, 0.06 pg/mL, 0.04 pg/mL, and 0.04 pg/mL for IFN-γ, IL-6, IL-10, and TNF-α, respectively.

Leukocyte subsets—The complete blood count with five-part differential was performed on the venous blood sample (collected in a lavender EDTA tube), stored at room temperature and processed within 4 hours of collection, at the Emory Medical Laboratory using the Beckman Coulter MAXM hematology analyzer (Fullerton, CA). It yielded absolute numbers of circulating basophils, eosinophils, neutrophils, monocytes, and lymphocytes.

White Blood Cell (WBC) dexamethasone (DEX) sensitivity—The procedure for whole blood DEX testing, originally described in the parent study protocol (Corwin et al., 2017), was adapted from previous work [19]. Restated here: 450 μl of whole blood diluted 10:1 with sterile saline was incubated with lipopolysaccharide (LPS; Difco, Augsburg, Germany; final concentration 30 ng/ml) along with increasing concentrations of DEX (Sigma, Deisenhofen, Germany; 10^{-8} to 10^{-4} M) in 24 well cell culture plates for 24 h at 37°C in 5% CO_2. Culture samples were centrifuged at 1000 × g for 10 min, the supernatants removed and aliquoted for storage at −80 °C until assayed for TNF-α, the indicator cytokine (Fisher Scientific, BD Cell Analysis, Atlanta, GA).

For analysis, we determined three values from these procedures. First, the white blood cells' ability to produce inflammatory cytokines was represented by TNF-α concentration in LPS-treated cultures that did not contain dexamethasone (or, Uninhibited TNF-α). Second, sensitivity of white blood cells to the anti-inflammatory properties of glucocorticoids was estimated by generating a dose-response curve for each subject. We then calculated the concentration of dexamethasone needed to diminish TNF-α production by 50% (or, DEX IC_{50}). DEX IC_{50} calculations were performed in GraphPad Prism 6.01 (San Diego, CA),

with mean determination coefficient >0.9. DEX IC_{50} is inversely related to glucocorticoid sensitivity [18], such that higher IC_{50} values indicate that more dexamethasone is needed to suppress TNF-α production by 50%. Thus, higher IC_{50} values also indicate that white blood cells are more resistant to anti-inflammatory signaling by glucocorticoids. Third, we examined suppressed cytokine production represented by TNF-α values in LPS-treated cultures after exposure to the maximum dexamethasone concentration (or, Max-inhibition TNF-α).

Statistical analyses

For each participant, we calculated the IC_{50} using a logistic curve fit function (mean determination coefficient of r^2>0.9). The resulting measure is independent of the absolute number of circulating monocytes.

We visualized the data using histograms and Q-Q plots. We log-10 transformed uninhibited TNF-α, DEX IC_{50}, and Post-Inhibition TNF-α values prior to analysis to stabilize variance and better approximate a normal distribution. We report univariate analyses of sociodemographic and biological characteristics as mean (standard deviation) or mean (interquartile range) for all variables we considered numeric, and as frequency (percent) for all categorical variables.

Continuous variables include: all *in vitro* measures of inflammation (Uninhibited TNF-α, DEX IC_{50}, and Post-Inhibition TNF-α), all serum measures of inflammation (CRP, IFN-γ, IL-6, IL-10, and circulating TNF-α), age, first-trimester BMI, and gestational age at blood draw. Categorical variables include: education, gestational diabetes status, hypertension status, insurance status, parity, and relationship status. Variable categories are discussed in our results and delineated in Table 1. We present first-trimester BMI categories in Table 1 in to facilitate interpretation according to clinical guidelines; it is later analyzed as a continuous variable Parity was included as both continuous and categorical variable in the analyses.

A unique purpose of this study was to describe DEX-mediated inhibition of the immune response in a relatively large population of pregnant African American women. Given this descriptive nature, we did not perform inferential tests comparing different within-race groups. We calculated associations between all pairwise combinations of numeric variables using the Pearson correlation coefficient. Bivariate associations between numeric and categorical variables were calculated using the independent sample Student t-test or analysis of variance (ANOVA). We report statistical significance at $p<.05$.

To estimate leukocyte cell type contribution to TNF-α *in vitro*, we used separate generalized linear models to regress Uninhibited TNF-α, DEX IC_{50}, and Max-inhibition TNF-α onto participant characteristics and leukocyte subsets. All participant characteristics were included in generalized linear models, regardless of their bivariate association with the dependent *in vitro* inflammatory marker in study data, given theoretical and/or empirical support for their role in immune activation in the literature. We performed stepwise regression, using the Schwarz Bayesian Information criterion [31], to reduce the full generalized linear models (i.e., models including all leukocyte subsets and participant characteristics) to just those variables most statistically predictive of the dependent *in vitro*

inflammatory marker. Where appropriate, we report standardized beta coefficients, which remove the units of measurement of independent and dependent variables. We also used stepwise regression to estimate the portion of variance in Uninhibited TNF-α explained by absolute monocyte count that is not already accounted for by the patient characteristics listed in Table 1.

RESULTS

Sample characteristics

At the time of this analysis, 485 women were enrolled in the parent study, of whom 408 with complete leukocyte subset and dexamethasone sensitivity variables (described below) were included in the present study. Of those 408 enrollees, we had complete serum inflammatory marker (CRP-reactive protein, IFN-γ, IL-6, IL-10, TNF-α) data for 184.

Demographic & health characteristics for the 408 pregnant African American women in this study are shown in Table 1. Characteristic dose response curves are shown in Figure 1. These individual dose-response curves (depicting the relationship between TNF-α (pg/ml) *in vitro* and the concentration of DEX needed to suppress the immune response after LPS stimulation at three points along the DEX IC_{50} distribution: within the lowest 10% of values, within one standard deviation of the average, and within the highest 10%.

Inflammatory markers, leukocyte subsets, and their associations

Descriptive statistics for their *in vitro* and serum inflammatory markers, and leukocyte subsets are outlined in Table 2. The median concentration of DEX needed to inhibit TNF-α production by 50% (i.e., DEX IC_{50}) was 8.8 nmol/L. The median concentration of TNF-α produced *in vitro* after LPS-stimulation was 623.9 pg/mL. Median max-inhibition TNF-α, or TNF-α after the maximum amount of DEX was administered, was 33.0 pg/mL. Median values for cytokines IFN-γ, IL-6, IL-10, TNF-α were within the wide range previously reported in early pregnancy [32]. Median CRP was 5.3 mg/L. Thirty-two percent of women (data not shown) had CRP values above 10 mg/L, an established clinical cutoff for systemic inflammation. Median leukocyte counts were within normal clinical reference ranges for pregnant women in their first trimester.

To understand the degree to which *in vitro* and serum inflammatory markers, and leukocyte subsets were associated with demographic and health characteristics, we performed bivariate tests (Tables S1 & S2). Uninhibited TNF-α was not significantly associated with the evaluated participant characteristics. DEX IC_{50} was also only significantly associated with parity ($p<0.05$), such that women who had three or more previous live births had higher IC_{50} values than women who had just one previous birth (Difference in means [95% CI]: 0.14 nmol/L [0.01, 0.27]). There were no other significant differences in mean IC_{50} between other parity groups. Max-inhibition TNF-α showed a weak, inverse linear correlation with first-trimester BMI ($r=-0.12$, $p<.05$), and was significantly associated with relationship status ($p<.01$). Mean max-inhibition TNF-α was higher among women who were unpartnered, as compared to women who were partnered (Difference in means [95% CI]: 0.23 pg/mL [0.06, 0.41]).

CRP moderately correlated with first-trimester BMI (r=0.52, p<.0001). Mean CRP was significantly higher among women with private versus public insurance (Difference in means [95% CI]: 0.40 mg/L [0.04–0.77]). IFN-γ was significantly higher among women without gestational diabetes than among women with gestational diabetes (Difference in means [95% CI]: 2.41 pg/mL [0.07, 4.74]). IL-6 was significantly higher among older women (r=0.2, p<0.05), women with higher first-trimester BMIs (r=0.34, p<.0001), among college educated (versus not college educated) women (Difference in means [95% CI]: 0.19 pg/mL [0.02, 0.40]), and among women with private (versus public) insurance (Difference in means [95% CI]: 0.32 pg/mL [0.07, 0.57]). Circulating TNF-α was higher among women who were receiving Medicaid versus the privately insured (Difference in means [95% CI]: 0.82 pg/mL [0.01, 1.64]). IL-10 was not significantly associated with any of the patient characteristics we investigated.

Basophil count also showed no significant association with patient characteristics. Both absolute eosinophils and absolute lymphocytes were weakly correlated with first-trimester BMI (r= 0.13, 0.16, respectively, p<.05). Eosinophil count was also correlated with gestational age at the time of blood draw (r=0.19, p<.01). Lymphocyte count was additionally associated with hypertension status, and was higher among women with any history of hypertension, as compared to women with no hypertensive conditions currently or in their past (Difference in means [95% CI]: 0.18 10^3/μL [0.01, 0.35]). Absolute monocytes showed the same relationship with hypertension (Difference in means [95% CI]: 0.08 10^3/μL [0.02, 0.13]). Monocyte count was also higher among nulliparous women as compared to women who had 2 previous live births (Difference in means [95% CI]: 0.09 10^3/μL [0.02, 0.17]). Neutrophil count was weakly, positively correlated with gestational age at blood draw (r=0.15, p<0.01).

In Table 3, we show linear correlations between the *in vitro* inflammatory markers and the remaining blood analytes. Most of the significant correlations (i.e., those with p<.05) were weak, with correlation coefficients less than ±0.30. All significant correlations with Uninhibited TNF-α were in the positive direction, while all significant correlations with DEX IC_{50} and Max-inhibition TNF-α were in the negative direction. We observed a moderately strong positive linear relationship between Uninhibited TNF-α and monocyte count (r = 0.40, p<.0001).

GC sensitivity by leukocyte subsets and participant characteristics

In regression on Uninhibited TNF-α adjusted only for age, as shown in Figure 2, monocytes and neutrophils contribute most, but in opposite directions (standardized $\beta_{monocytes}$=0.59, p<.0001; standardized $\beta_{neutrophils}$=−0.40, p<.0001). In age-adjusted regression on DEX IC_{50}, standardized regression coefficients for all leukocyte subsets are similar in magnitude (β≤0.10, all non-intercept p-values>.05).

Results from multivariable regression models, which included all evaluated participant characteristics and leukocyte subsets, are presented in Table 4. Together, all patient characteristics shown in Table 1 and all leukocyte subsets collectively explain about 30% of the variance in Uninhibited TNF-α. In a model reduced by stepwise selection, only monocyte and neutrophil counts are maintained, and explain 27% of the variance in

Uninhibited TNF-α. Only 7.2% of the variance in DEX IC_{50} is explained by patient characteristics and leukocyte subsets. After stepwise regression, only neutrophils remain in the model; they explain 1.7% of the variance in DEX IC_{50}. There were no significant multivariable relationships between the independent variables and Max-inhibition TNF-α; those data are not shown.

To determine the unique contribution of absolute monocyte count to Uninhibited TNF-α, we used stepwise regression, illustrated in Figure 3. In Model 1, participant characteristics not significantly associated with monocytes (i.e., age, education, gestational age at blood draw, gestational diabetes, insurance status, and relationship status; Tables S1 & S2) explained 2.2% of the variance in Uninhibited TNF-α. The percent of variance explained increased to 16.4% in Model 2, which included monocytes and Model 1's participant characteristics. Finally, in Model 3, when parity and hypertension (the health variables bivariately associated with monocyte count; Tables S1 & S2) were added to the independent variables in Model 2, the percent of variance explained increased marginally to 17.4%.

DISCUSSION

In the current study we report a detailed laboratory assessment of leukocyte glucocorticoid sensitivity, as well as cytokine relationships with sociodemographics, in >400 pregnant African American women. Despite the central role of diminished GC sensitivity in most biopsychosocial models of chronic stress (and its effects on immune function), most studies have not examined GC sensitivity directly by measuring the dose-response relationship between activation of the glucocorticoid receptor and inflammatory output. In the context of pregnancy, the data are even more scant, especially among African Americans who experience a disproportionate burden of chronic stress and pregnancy complications. Thus our large-scale study with statistical adjustment for confounders will serve as an anchor point for future studies, and has several key findings and broad implications for obstetrics and psychoneuroimmunology.

GC sensitivity and relationship to leukocyte subsets

There is a long-standing belief that monocytes are responsible for the production of proinflammatory cytokines after LPS stimulation, and that glucocorticoids must be acting through these cells to suppress cytokine production [33–38]. Until now, this has not been tested extensively in pregnant populations. Thus, comparison of our results with similar studies is limited. Still, our data largely support the primary role of monocytes in production of TNF, although our findings present several important nuances. As expected, monocyte counts were positively correlated with Uninhibited TNF-α (i.e., the top of the dose-response curve) because this value represents the production of TNF-α in the absence of the inhibitory influence of dexamethasone. Prior *ex vivo* studies often express TNF-α as a ratio per the number of monocytes, implying that monocytes are the sole source of *ex vivo* production of TNF-α, and that there is a direct one-to-one relationship between TNF production and monocyte number [39–41]. However, in African American women's early pregnancy, we found that the number of monocytes could only explain 14% of the interindividual variation in TNF-α production in the absence of glucocorticoids to suppress the immune response.

Surprisingly, none of the sociodemographic variables (e.g. maternal age, BMI, insurance status, education) were found to influence the *in vitro* production of TNF in response to LPS. Denney et al. [42] examined the response to LPS in 45 women during their first trimester of pregnancy and found roughly similar levels of TNF (median: 591 pg/ml) to our findings (median: 624 pg/ml). That study did not test GC sensitivity, though it did have good representation of African American women [42].

Somewhat unexpectedly, we found that basophils and eosinophils were also correlated with Uninhibited TNF-α. This is a weak correlation, but still significant. We also found that monocyte count had a weak negative correlation with the DEX IC_{50}, suggesting that those individuals with more monocytes in their blood might require less dexamethasone to suppress TNF-α. Hence, their available monocytes may be relatively sensitive to glucocorticoids. Conversely, an individual with a lower monocyte count would have fewer such cells to produce TNF-α, but the available cells would be relatively resistant to glucocorticoid suppression. However, in adjusted regression models, the monocyte count had relatively little association with the DEX IC_{50}. A similar negative association was found with neutrophils, implying that those with more neutrophils are relatively sensitive to dexamethasone as compared with those women with lower neutrophils counts.

This raises the question of whether it is necessary or advantageous to adjust for monocytes and neutrophils in studies that only use one concentration of dexamethasone to impute GC resistance. As shown by our examples of full dose- response curves for dexamethasone, both the IC_{50} and shape of the curve can vary between individuals. Since these two parameters are interrelated, a simple substitution of one concentration of dexamethasone will yield less complete data than a full dose response curve. However, at the IC_{50} for dexamethasone, the monocyte count makes only a minor contribution to the variance.

Relationship between circulating and in vitro stimulated cytokines

There has been substantial discussion concerning the differences between circulating levels of cytokines, and levels that are stimulated by a pathogen or immune-stimulant such as LPS [43]. *A priori*, we might expect that individuals with higher levels of circulating proinflammatory molecules (CRP, IL-6, TNF-α) would be in a relatively higher inflammatory state, and that *in vitro* stimulation of immune cells would further reveal this state. Thus, we would expect those proinflammatory molecules to be positively correlated with the top of the dose-response curve (high *in vitro* TNF). However, only CRP and IL-6 show this positive correlation. Surprisingly, circulating TNF-α itself was not significantly correlated with *in vitro* TNF-α. However, both IFN-γ and IL-10 were positively correlated with *in vitro* TNF-α production.

CRP in pregnancy among African Americans

In our study of socioeconomically diverse pregnant African American women, median first trimester CRP is 5.3 mg/L (IQR: 2.0–13.6). While this value is above previously published thresholds for sub-clinical inflammation, other studies find similar results, suggesting increased systemic inflammation among pregnant and non-pregnant African American women. In a large (n=2,749) population-based study of non-pregnant Black and White

participants, Black participants had significantly higher CRP as compared to White participants (median: 3.0 vs. 2.3 mg/L; $p < 0.001$) [44]. Furthermore, the highest percentage of those with CRP >3mg/L was found among Black women (vs White women, Black men, and White men) [44]. In an even larger population-based longitudinal study of over 26,000 participants, 49% of Black participants (compared to 35% of White participants) had CRP >3mg/L [45]. These results suggest racial differences in CRP among non-pregnant Black individuals, whose CRP profile may continue to differ from that of White counterparts during pregnancy, which itself acts as an inflammatory stressor [46].

In one analysis aimed at longitudinally characterizing CRP in a diverse group of pregnant women, median CRP for pregnant African American women with gestational ages < 14 weeks (as in our sample) was 7.68 mg/L—even higher than in the present study [46]. Median CRP among pregnant white women in that study was 2.59 mg/L [46]. There was also a strong association between elevated serum CRP (i.e. above the 75th percentile, 15.7 mg/L) and African American race (OR [95%CI]: 2.1 [1.3–3.3]), and other sociodemographic factors (i.e. socioeconomic status was indexed by insurance type in our study). Additionally, unlike CRP for White women which showed a slight upward trend with increasing gestational age, CRP for African American women remained high and relatively flat throughout pregnancy [46].

Other analyses have found elevated CRP among pregnant women who deliver preterm [24, 47–50]. There is a growing literature supporting a role for harmful social and structural factors in the association between African American race and preterm birth [50–52]. Thus, we could hypothesize that those same factors adversely influence inflammatory markers in pregnancy to some degree whether or not preterm delivery eventually occurs downstream.

Indeed, the biology of preterm birth among African American women (including mechanisms acting through the maternal immune system) may be dependent on exposure to racial discrimination [1]. A 16-study meta-analysis found a small association between racial discrimination and cortisol output across studies, but states that this is likely due to heterogeneity in the ways in which chronic stress impacts HPA activity based on the individual and/or stressor [53]. Across the 16 studies included, sample characteristics (including race/ethnicity and sex) varied, which may have obscured subpopulation-specific findings. In a novel study on leukocyte glucocorticoid sensitivity and racial discrimination among 91 African American women, Gillespie and Anderson [1] found that decreased GC sensitivity was associated with a higher frequency of experiences with racial discrimination. Experiences of discrimination have also been linked to preterm birth among African American women [54].

The purpose of the present study was not to examine the relationship between CRP, cytokines, and any type of perceived stress explicitly. We observed correlations in support of and against stress leading to GC suppression; thus, further study is needed to clarify those complex biopsychosocial relationships. In addition to CRP, the other soluble pro-inflammatory molecules we measured (IL-6, TNF-α) have also been associated with psychological stress and depression during pregnancy, including African American women [7, 55]. If we consider some of the possible proxy variables for chronic stress such as public

insurance, we found that CRP & IL-6 were higher among women with private insurance, but TNF-α was higher among women receiving Medicaid. Thus, in the current study we did not make specific inferences concerning these pro-inflammatory molecules and stress exposure. Moreover, our results in this specific cohort need to be considered in the context of a substantial (and sometimes inconsistent) literature on the relationship of stress-associated changes in HPA-axis hormones and cytokines in non-pregnant individuals.

Additionally, prospective data in African American women with healthy versus adverse outcomes would inform our understanding of the potential role of immune-dysregulation in racial disparities in birth outcomes among women at high risk for chronic stress and immune activation. Additionally, investigation into the role of early life experiences [56] and coping mechanisms developed over the life course [53] may offer insight into protective factors.

Strengths of the current study

Despite the need for more comprehensive information on maternal immune regulation in pregnancy, there have been few large-scale studies of glucocorticoid receptor sensitivity in pregnant women that use a full stimulus dose-response curve to represent GC receptor binding and downstream signaling processes. This study is among the first of its kind to examine this phenomenon in a large sample of pregnant African American women, a population at disproportionate risk. The few prior studies in human pregnancy have used either surrogate or indirect tests of glucocorticoid sensitivity, such as the ratio of pro-inflammatory to anti-inflammatory cytokines in relation to cortisol [57].

While there are some data to support the WBC types responsible for producing pro-inflammatory cytokines *in vitro* [58], the relative contribution of each of the leukocyte types has not been systematically examined in healthy pregnancy, nor have many studies focused on African American women. Studies in non-pregnant populations confirm that *ex vivo* stimulation of whole blood with dexamethasone (DEX) can indeed be used to assess cytokine production and glucocorticoid sensitivity [59–62]. However, there had not been sufficient information on how the relative cell type composition may influence points along the full DEX dose-response curve. We have demonstrated that while monocytes may be the main source of TNF-α [36, 37, 63], other leukocyte cell types (which also have glucocorticoid receptors) may also interact in a paracrine manner to influence the glucocorticoid-sensitive responses of monocytes.

For our *in vitro* study, we measured TNF as the index inflammatory cytokine in accordance with standard methodology [19]. In unrelated studies among non-pregnant individuals, we find a similar dose-response curve for DEX when using IL-6 as the index cytokine. Future studies could also examine immunoregulatory in anti-inflammatory cytokines (e.g. IL10), though this was cost prohibitive in the current study.

Limitations

Given our focus on responses to LPS, and thus on the GC sensitivity of the innate immune response, we did not examine adaptive immunity. We collected blood only at the end of the first trimester and did not attempt to examine longitudinal changes in either LPS responses or glucocorticoid sensitivity. One prior study found a slight increase in LPS stimulated TNF

across gestation [42]. We did not attempt to compare African American women to White women, though our study represents the most comprehensive analysis of GC resistance in early pregnancy to date, regardless of race. Finally, at the time of analysis, CRP and cytokine assays were available for a portion (n=184) of the total study population.

Conclusions

Our comprehensive, descriptive study of GC resistance during early pregnancy and correlations with leukocyte cell types in circulating cytokines should serve as one important, hypothesis-generating anchor to the growing recognition of interindividual variation in HPA axis regulation and inflammatory responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

The authors are grateful to the women who generously agree to participate in our studies. We are also grateful to the research coordinators who interface with the participating women to carefully collect research data and to the clinical staff at Emory Midtown and Grady Hospitals for their support in recruiting participants. We thank Danielle Chou for technical help.

Funding Statement: This study was supported in part by the National Institutes of Health, National Institute of Nursing Research [R01NR014800], National Institute on Minority Health and Health Disparities [R01MD009064], and the Office of the Director [UH3OD023318].

Abbreviations

BMI	body mass index
CRP	C-reactive protein
DEX	dexamethasone
GC	glucocorticoid
HPA	hypothalamic-pituitary-adrenal
IFN	interferon
IL	interleukin
LPS	lipopolysaccharide
PTB	preterm birth
TNF	tumor necrosis factor
WBC	white blood cell

References

1. Gillespie SL, Anderson CM. Racial discrimination and leukocyte glucocorticoid sensitivity: Implications for birth timing. Social Science & Medicine. 2018;216:p.114–123. [PubMed: 30309686]
2. Velez DR, Fortunato SJ, Morgan N, et al. Patterns of cytokine profiles differ with pregnancy outcome and ethnicity. Hum. Reprod. 2008;23(8):p.1902–1909. [PubMed: 18487217]
3. Brou L, Almli L, Pearce B, et al. Dysregulated biomarkers induce distinct pathways in preterm birth. BJOG: An International Journal of Obstetrics & Gynaecology. 2012;119(4):p.458–473. [PubMed: 22324919]
4. Corwin EJ, Guo Y, Pajer K, et al. Immune dysregulation and glucocorticoid resistance in minority and low income pregnant women. Psychoneuroendocrinology. 2013;38(9):p.1786–1796. [PubMed: 23541234]
5. Holsboer F The corticosteroid receptor hypothesis of depression. Neuropsychopharmacology. 2000;23(5):p.477–501. [PubMed: 11027914]
6. Pariante CM, Lightman SL. The hpa axis in major depression: Classical theories and new developments. Trends Neurosci. 2008;31(9):p.464–468. [PubMed: 18675469]
7. Coussons-Read ME. Stress and immunity in pregnancy. The Oxford Handbook of Psychoneuroimmunology. 2012:p.3.
8. Christian LM. Psychoneuroimmunology in pregnancy: Immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. Neurosci. Biobehav. Rev 2012;36(1):p.350–361. [PubMed: 21787802]
9. Ruiz RJ, Fullerton J, Dudley DJ. The interrelationship of maternal stress, endocrine factors and inflammation on gestational length. Obstet Gynecol Surv. 2003;58(6):p.415–28. [PubMed: 12775946]
10. Parker VJ, Douglas AJ. Stress in early pregnancy: Maternal neuro-endocrine-immune responses and effects. J. Reprod. Immunol 2010;85(1):p.86–92. [PubMed: 20079933]
11. Wadhwa PD, Culhane JF, Rauh V, et al. Stress, infection and preterm birth: A biobehavioural perspective. Paediatric and Perinatal Epidemiology. 2001;15 Suppl 2:p.17–29. [PubMed: 11520397]
12. Hogue CJ, Bremner JD. Stress model for research into preterm delivery among black women. American Journal of Obstetrics and Gynecology. 2005;192(5, Supplement):p.S47–S55. [PubMed: 15891712]
13. Kramer MR, Hogue CJ, Dunlop AL, Menon R. Preconceptional stress and racial disparities in preterm birth: An overview. Acta obstetricia et gynecologica Scandinavica. 2011;90(12):p.1307–16. [PubMed: 21446927]
14. Cassidy-Bushrow AE, Peters RM, Johnson DA, Templin TN. Association of depressive symptoms with inflammatory biomarkers among pregnant african-american women. J. Reprod. Immunol 2012;94(2):p.202–209. [PubMed: 22386525]
15. Latendresse G The interaction between chronic stress and pregnancy: Preterm birth from a biobehavioral perspective. J Midwifery Womens Health. 2009;54(1):p.8–17. [PubMed: 19114234]
16. Schetter CD. Stress processes in pregnancy and preterm birth. Curr. Dir. Psychol. Sci 2009;18(4):p.205–209.
17. Christian LM. Stress and immune function during pregnancy: An emerging focus in mind-body medicine. Current directions in psychological science. 2015;24(1):p.3–9. [PubMed: 25745279]
18. Leistner C, Menke A. How to measure glucocorticoid receptor's sensitivity in patients with stress-related psychiatric disorders. Psychoneuroendocrinology. 2018;91:p.235–260. [PubMed: 29449045]
19. Franchimont D, Louis E, Dewé W, et al. Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures. Regul. Pept 1998;73(1):p.59–65. [PubMed: 9537674]
20. Christian LM, Glaser R, Porter K, Iams JD. Stress-induced inflammatory responses in women: Effects of race and pregnancy. Psychosom. Med 2013;75(7):p.658. [PubMed: 23873713]

21. Giurgescu C, Engeland CG, Templin TN, et al. Racial discrimination predicts greater systemic inflammation in pregnant african american women. Appl. Nurs. Res 2016;32:p.98–103. [PubMed: 27969060]
22. Allen AM, Thomas MD, Michaels EK, et al. Racial discrimination, educational attainment, and biological dysregulation among midlife african american women. Psychoneuroendocrinology. 2019;99:p.225–235. [PubMed: 30286445]
23. Thames AD, Irwin MR, Breen EC, Cole SW. Experienced discrimination and racial differences in leukocyte gene expression. Psychoneuroendocrinology. 2019;106:p.277–283. [PubMed: 31029930]
24. Pearce BD, Grove J, Bonney EA, et al. Interrelationship of cytokines, hypothalamic-pituitary-adrenal axis hormones, and psychosocial variables in the prediction of preterm birth. Gynecol Obstet Invest. 2010;70(1):p.40–46. [PubMed: 20160447]
25. Giurgescu C, Sanguanklin N, Engeland CG, et al. Relationships among psychosocial factors, biomarkers, preeclampsia, and preterm birth in african american women: A pilot. Applied Nursing Research. 2015;28(1):p.e1–e6. [PubMed: 25282477]
26. Katz E, Stowe Z, Newport DJ, et al. Regulation of mrna expression encoding chaperone and co-chaperone proteins of the glucocorticoid receptor in peripheral blood: Association with depressive symptoms during pregnancy. Psychol. Med 2012;42(5):p.943–956. [PubMed: 21995950]
27. Menon R, Velez DR, Thorsen P, et al. Ethnic differences in key candidate genes for spontaneous preterm birth: Tnf-α and its receptors. Hum. Hered 2006;62(2):p.107–118. [PubMed: 17047334]
28. Menon R, Thorsen P, Vogel I, et al. Increased bioavailability of tnf-α in african americans during in vitro infection: Predisposing evidence for immune imbalance. Placenta. 2007;28(8–9):p.946–950. [PubMed: 17517432]
29. Azizieh FY, Raghupathy RG. Tumor necrosis factor-α and pregnancy complications: A prospective study. Med. Princ. Pract 2015;24(2):p.165–170. [PubMed: 25501617]
30. Corwin EJ, Hogue CJ, Pearce B, et al. Protocol for the emory university african american vaginal, oral, and gut microbiome in pregnancy cohort study. BMC Pregnancy Childbirth. 2017;17(1):p.161. [PubMed: 28571577]
31. Schwarz G Estimating the dimension of a model. The annals of statistics. 1978;6(2):p.461–464.
32. Holtan SG, Chen Y, Kaimal R, et al. Growth modeling of the maternal cytokine milieu throughout normal pregnancy: Macrophage-derived chemokine decreases as inflammation/counterregulation increases. Journal of immunology research. 2015;2015.
33. Guha M, Mackman N. Lps induction of gene expression in human monocytes. Cell. Signal 2001;13(2):p.85–94. [PubMed: 11257452]
34. Plevin RE, Knoll M, McKay M, Arbabi S, Cuschieri J. The role of lipopolysaccharide structure in monocyte activation and cytokine secretion. Shock (Augusta, Ga.). 2016;45(1):p.22–27.
35. Steer JH, Kroeger KM, Abraham LJ, Joyce DA. Glucocorticoids suppress tumor necrosis factor-α expression by human monocytic thp-1 cells by suppressing transactivation through adjacent nf-κb and c-jun-activating transcription factor-2 binding sites in the promoter. J. Biol. Chem 2000;275(24):p.18432–18440. [PubMed: 10748079]
36. Wirtz PH, Känel Rv, Frey K, Ehlert U, Fischer JE. Glucocorticoid sensitivity of circulating monocytes in essential hypertension. Am. J. Hypertens 2004;17(6):p.489–494. [PubMed: 15177520]
37. Cepika AM, Bendelja K, Vergles JM, et al. Monocyte response to lps after exposure to corticosteroids and chloroquine with implications for systemic lupus erythematosus. Scand. J. Immunol 2010;72(5):p.434–443. [PubMed: 21039738]
38. Varga G, Ehrchen J, Brockhausen A, et al. Immune suppression via glucocorticoid-stimulated monocytes: A novel mechanism to cope with inflammation. The Journal of Immunology. 2014;193(3):p.1090–1099. [PubMed: 24990080]
39. Kox M, de Kleijn S, Pompe JC, et al. Differential ex vivo and in vivo endotoxin tolerance kinetics following human endotoxemia. Crit. Care Med 2011;39(8):p.1866–1870. [PubMed: 21494102]
40. Klimiec E, Kowalska K, Pasinska P, et al. Reduced release of tnfα and ip-10 after ex vivo blood stimulation with endotoxin is associated with poor outcome after stroke. Cytokine. 2018;102:p.51–54. [PubMed: 29275013]

41. Kowalska K, Klimiec E, Weglarczyk K, et al. Reduced ex vivo release of pro-inflammatory cytokines and elevated plasma interleukin-6 are inflammatory signatures of post-stroke delirium. J. Neuroinflammation 2018;15(1):p.111. [PubMed: 29669581]
42. Denney JM, Nelson EL, Wadhwa PD, et al. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine. 2011;53(2):p.170–177. [PubMed: 21123081]
43. Menon R, Torloni MR, Voltolini C, et al. Biomarkers of spontaneous preterm birth: An overview of the literature in the last four decades. Reproductive Sciences. 2011;18(11):p.1046–1070. [PubMed: 22031189]
44. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in c-reactive protein levels. J. Am. Coll. Cardiol 2005;46(3):p.464–469. [PubMed: 16053959]
45. Evans CR, Long DL, Howard G, et al. C-reactive protein and stroke risk in blacks and whites: The reasons for geographic and racial differences in stroke cohort. Am. Heart J 2019;217:p.94–100. [PubMed: 31520899]
46. Picklesimer AH, Jared HL, Moss K, et al. Racial differences in c-reactive protein levels during normal pregnancy. American journal of obstetrics and gynecology. 2008;199(5):p.523–e1. [PubMed: 18539258]
47. Pitiphat W, Gillman MW, Joshipura KJ, et al. Plasma c-reactive protein in early pregnancy and preterm delivery. Am. J. Epidemiol 2005;162(11):p.1108–1113. [PubMed: 16236995]
48. Vecchié A, Bonaventura A, Carbone F, et al. C-reactive protein levels at the midpregnancy can predict gestational complications. Biomed Res Int. 2018;2018:p.1070151–1070151. [PubMed: 30533423]
49. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum c-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. Clin Biochem. 2007;40(5–6):p.330–5. [PubMed: 17289011]
50. Borders AE, Wolfe K, Qadir S, et al. Racial/ethnic differences in self-reported and biologic measures of chronic stress in pregnancy. J. Perinatol 2015;35(8):p.580–4. [PubMed: 25789817]
51. Prather C, Fuller TR, Marshall KJ, Jeffries WLt. The impact of racism on the sexual and reproductive health of african american women. Journal of women's health (2002). 2016;25(7):p.664–71.
52. Slaughter-Acey JC, Sealy-Jefferson S, Helmkamp L, et al. Racism in the form of micro aggressions and the risk of preterm birth among black women. Annals of epidemiology. 2016;26(1):p.7–13.e1. [PubMed: 26549132]
53. Korous KM, Causadias JM, Casper DM. Racial discrimination and cortisol output: A meta-analysis. Social Science & Medicine. 2017;193:p.90–100. [PubMed: 29028560]
54. Alhusen JL, Bower KM, Epstein E, Sharps P. Racial discrimination and adverse birth outcomes: An integrative review. J Midwifery Womens Health. 2016;61(6):p.707–720. [PubMed: 27737504]
55. Christian LM, Franco A, Glaser R, Iams J. Depressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. Brain, Behav., Immun 2009;23(6):p.750. [PubMed: 19258033]
56. Hantsoo L, Jašarević E, Criniti S, et al. Childhood adversity impact on gut microbiota and inflammatory response to stress during pregnancy. Brain. Behav. Immun 2019;75:p.240–250. [PubMed: 30399404]
57. Saito S, Sakai M, Sasaki Y, et al. Quantitative analysis of peripheral blood th0, th1, th2 and the th1:Th2 cell ratio during normal human pregnancy and preeclampsia. Clin. Exp. Immunol 1999;117(3):p.550–555. [PubMed: 10469061]
58. Beckmann I, Efraim SB, Vervoort M, Visser W, Wallenburg HC. Tumor necrosis factor-α in whole blood cultures of preeclamptic patients and healthy pregnant and nonpregnant women. Hypertens. Pregnancy. 2004;23(3):p.319–329. [PubMed: 15617632]
59. Burnsides C, Corry J, Alexander J, et al. Ex vivo stimulation of whole blood as a means to determine glucocorticoid sensitivity. Journal of inflammation research. 2012;5:p.89. [PubMed: 22952414]
60. De Groote D, Zangerlé P-F, Gevaert Y, et al. Direct stimulation of cytokines (il-1β, tnf-α, il-6, il-2, ifn-γ and gm-csf) in whole blood. I. Comparison with isolated pbmc stimulation. Cytokine. 1992;4(3):p.239–248. [PubMed: 1498259]

61. Ray CA, Dumaual C, Willey M, et al. Optimization of analytical and pre-analytical variables associated with an ex vivo cytokine secretion assay. J. Pharm. Biomed. Anal 2006;41(1):p.189–195. [PubMed: 16321496]

62. Al-Hanbali M, Ali D, Bustami M, et al. Epicatechin suppresses il-6, il-8 and enhances il-10 production with nf-κb nuclear translocation in whole blood stimulated system. Neuroendocrinology Letters. 2009;30(1).

63. Wirtz PH, von Känel R, Rohleder N, Fischer JE. Monocyte proinflammatory cytokine release is higher and glucocorticoid sensitivity is lower in middle aged men than in women independent of cardiovascular risk factors. Heart. 2004;90(8):p.853–858. [PubMed: 15253951]

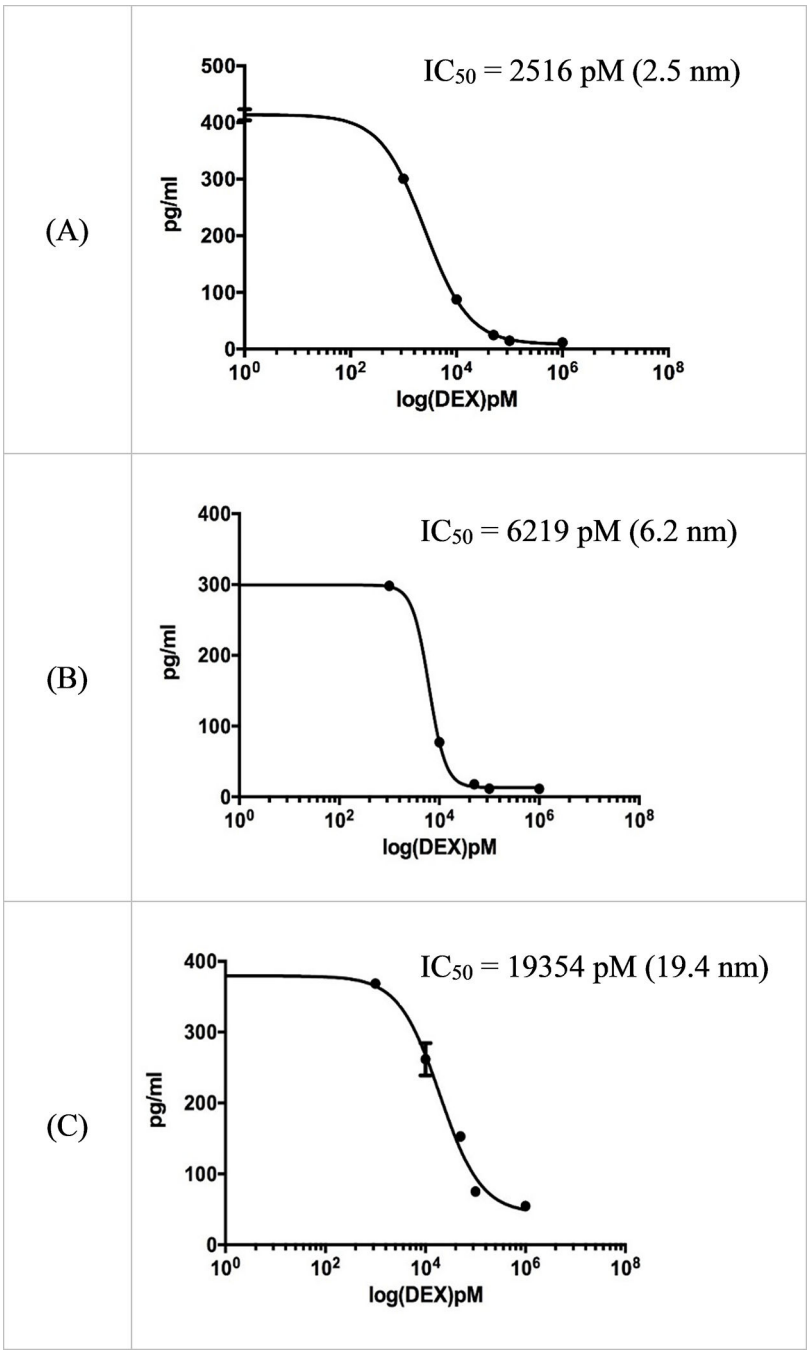

Fig. 1.

In vitro dexamethasone suppression test dose-response (D/R) curves.

The y-axes represent TNF-α (pg/ml) present *in vitro* after LPS-stimulation. The x-axes represent the log of the amount of DEX administered to suppress the immune response. Using a log-linear D/R curve, the DEX IC_{50} is an established parameter of glucocorticoid resistance, and a measure of the concentration of DEX needed to inhibit 50% of the LPS-induced TNF-α production. The top of the D/R curve represents TNF-α production when no DEX is present, or Uninhibited TNF-α. The bottom of the D/R curve represents TNF-α

production after the maximum DEX administration, or Post-Inhibition TNF-α. (**A**) D/R curve for a study participant with a DEX IC_{50} value in the lowest 10%, (**B**) D/R curve for a study participant with a DEX IC_{50} value within 1 standard deviation of the average, (**C**) D/R curve for a study participant with a DEX IC_{50} value in the highest 10%. Note: these D/R curves were chosen for illustrative purposes, and may not represent the full scope of curve shapes observed.

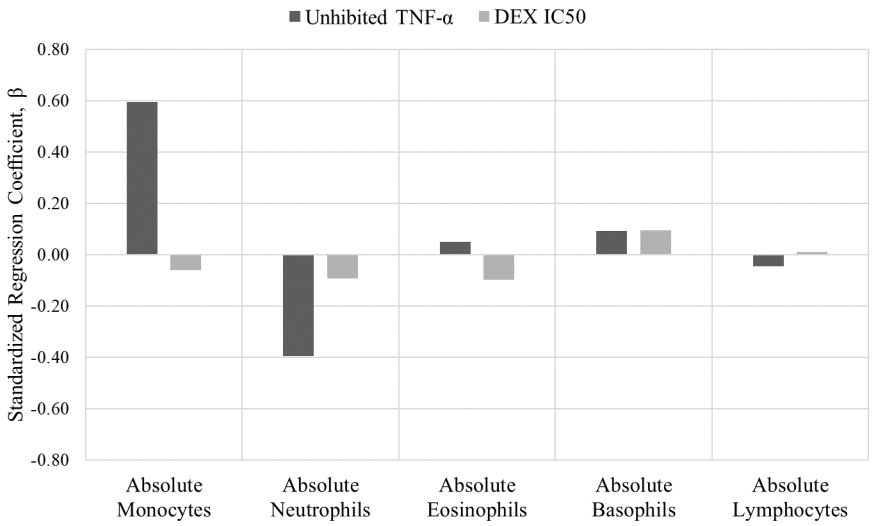

Fig. 2.

Age-adjusted regression on Uninhibited TNF-α & DEX IC_{50} by leukocyte subsets.

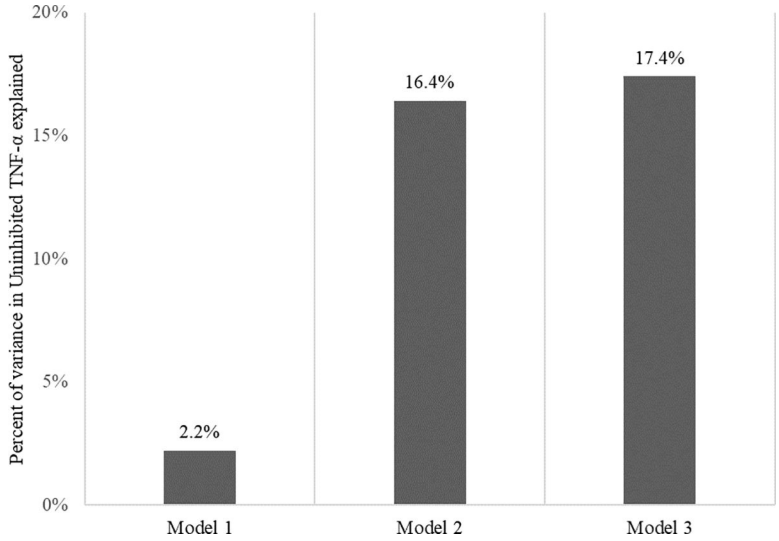

Fig. 3.

Stepwise regression models predicting Uninhibited TNF-α.

Model 1 includes only participant characteristics that were not independently associated with monocytes, i.e., age, education, gestational age at blood draw, gestational diabetes, insurance status, and relationship status. We exclude parity & hypertension, which were associated with monocytes (Tables S1 & S2), to reduce multicollinearity in the model, and to better estimate the contribution of monocytes alone. Model 2 includes the same participants characteristics included in Model 1, as well as absolute monocytes. In model 3, parity & hypertension were added in. The difference in the percent of variance explained by variables in the models 1 and 2 is 14.2%, suggesting an important role for monocutes in predicting uninhibited TNF-α.

Table 1.

Characteristics of pregnant African American women in their first trimester (n=408)

Characteristic	Study Participants (N = 408) n (%)
Age (years)	
Overall, Mean (SD)	24.8 (4.6)
18–24	219 (53.7)
25–35	189 (46.3)
First-trimester BMI[a, b]	
Overall, Mean (SD)	28.3 (7.7)
<18.5	14 (3.7)
18.5 – <25	155 (40.6)
25 – <30	77 (20.2)
30+	136 (35.6)
Education[a, c]	
College educated	191 (46.9)
Not college educated	216 (53.1)
Gestational age at blood draw (weeks) [a]	
Overall, Mean (SD)	11.4 (2.7)
Gestational diabetes	
Yes	11 (3.0)
No	359 (97.0)
Hypertension[a, d]	
None	310 (83.8)
Chronic hypertension	33 (8.9)
Gestational hypertension	5 (1.4)
Preeclampsia	20 (5.4)
Superimposed preeclampsia	2 (0.5)
Insurance type[a, e]	
Public	323 (79.2)
Private	85 (20.8)
Parity[a]	
Overall, Mean (SD)	0.9 (1.0)
0	187 (47.8)
1	104 (26.6)
2	63 (16.1)
3 or more	37 (9.5)

Characteristic	Study Participants (N = 408) n (%)
Relationship status[a, f]	
Partnered	315 (78.6)
Not partnered	86 (21.4)

Note. First-trimester BMI = body mass index (BMI) at first prenatal care study visit

[a]Variable (n, % missing): first-trimester BMI (26, 6.4), education (1, 0.2), gestational age at blood draw (26, 6.4), gravidity (26, 6.4), hypertension (38, 9.3), parity (17, 4.2), relationship status (7, 1.7); other variables are complete

[b]First-trimester BMI was categorized according to clinical guidelines: Underweight (<18.5 kg/m^2), Normal Weight (18.5 – <25), Overweight(25 – <30), Obese (≥30).

[c]Participants who reported completing “at least some college” were categorized as “College educated.” Not college educated participants are those who have not completed any college.

[d]Hypertension status reflects diagnosis at the tie of medical abstraction, if any. All categories are mutually exclusive. Multiple diagnoses are shown in Table 1 for descriptive purposes. Later analyses including hypertension status use a dichotomous variable (i.e., any hypertensive diagnosis vs no hypertensive diagnosis).

[e]The “Public insurance” category includes participants enrolled in Medicaid.

[f]Those who reported being married or in a relationship, regardless of cohabitation status, were categorized as “Partnered.” Those who reported being single were categorized as “Not partnered.”

Table 2.

Blood analytes

	Raw means (SD)	Raw medians (IQR)
***In vitro* inflammatory markers**		
Uninhibited TNF-α (pg/mL)	714.7 (450.7)	623.9 (402.3–952.4)
DEX IC_{50} (nmol/L)	9.8 (5.8)	8.8 (5.9–11.8)
Max-inhibition TNF-α (pg/mL)	56.0 (66.2)	33.0 (18.2–72.2)
Inflammatory markers[a]		
CRP (mg/L)	9.4 (11.0)	5.3 (2.0–13.0)
IFN-γ (pg/mL)	5.9 (l0.9)	3.0 (2.0–4.5)
IL-6 (pg/mL)	0.9 (0.7)	0.7 (0.5–1.1)
IL-10 (pg/mL)	0.5 (0.6)	0.4 (0.3–0.5)
Circulating TNF-α (pg/mL)	3.2 (2.4)	2.4 (2.0–3.3)
Leukocyte Subsets		
Absolute Basophil (10^3/μL)	0.02 (0.02)	0.02 (0.01–0.02)
Absolute Eosinophil (10^3/μL)	0.1 (0.1)	0.1 (0.1–0.1)
Absolute Lymphocyte (10^3/μL)	1.9 (0.6)	1.8 (1.5–2.2)
Absolute Monocyte (10^3/μL)	0.5 (0.2)	0.5 (0.4–0.6)
Absolute Neutrophil (10^3/μL)	4.8 (2.0)	4.5 (3.4–6.0)

[a]Variable (n, % missing) for all inflammatory markers: 224 (54.9)

Table 3.

Bivariate associations of *in vitro* makers of inflammation with leukocyte subsets and inflammatory markers (Correlation coefficient, r)

	Uninhibited TNF-α (pg/mL)	DEX IC_{50} (nmol/L)	Post-inhibition TNF-α (pg/mL)
Absolute Basophils (10^3/μL)	**0.15** **	0.06	0.03
Absolute Eosinophils (10^3/μL)	**0.11** *	**−0.10** *	−0.07
Absolute Lymphocytes (10^3/μL)	0.06	−0.02	−0.01
Absolute Monocytes (10^3/μL)	**0.40** ***	**−0.11** *	0.01
Absolute Neutrophils (10^3/μL)	−0.08	**−0.13** *	**−0.13** **
CRP (mg/L)	**0.15** *	0.12	−0.02
IFN-γ (pg/mL)	**0.15** *	−0.04	0.1
IL-6 (pg/mL)	**0.18** *	−0.03	−0.11
IL-10 (pg/mL)	**0.25** **	0.04	0.08
TNF-α (pg/mL)	0.08	−0.12	0.003

* p<.05

** p<.01

*** p<.0001

Table 4.

Multivariable regression on Uninhibited TNF-α & DEX IC_{50} by leukocyte subsets & patient characteristics

Dependent variable: Uninhibited TNF-α				
Independent variable	**1. All leukocyte types and participant characteristics**[a]		**2. Independent variables remaining after stepwise selection**	
	R^2= 30.2		R^2=27.0%	
	Standardized Regression Coefficient, β (±SEM)	p	Standardized Regression Coefficient, β (±SEM)	p
Absolute Basophil (10^3/μL)	0.09 (±0.82)	0.05	--	--
Absolute Eosinophil (10^3/μL)	0.05 (±0.16)	0.32	--	--
Absolute Lymphocyte (10^3/μL)	−0.04 (±0.02)	0.41	--	--
Absolute Monocyte (10^3/μL)	0.58 (±0.08)	<.0001	0.59 (±0.08)	<.0001
Absolute Neutrophil (10^3/μL)	−0.41 (±0.01)	<.0001	−0.40 (±0.01)	<.0001
Dependent variable: DEX IC_{50}				
Independent variable	**1. All leukocyte types and participant characteristics**[a]		**2. Independent variables remaining after stepwise selection**	
	R^2= 7.2%		R^2=1.7%	
	Standardized Regression Coefficient, β (±SEM)	p	Standardized Regression Coefficient, β (±SEM)	p
Absolute Basophil (10^3/μL)	0.10 (±0.90)	0.08	--	--
Absolute Eosinophil (10^3/μL)	−0.10 (±0.18)	0.06	--	--
Absolute Lymphocyte (10^3/μL)	0.01 (±0.02)	0.86	--	--
Absolute Monocyte (10^3/μL)	−0.11 (±0.09)	0.10	--	--
Absolute Neutrophil (10^3/μL)	−0.07 (±0.01)	0.25	−0.13 (±0.01)	0.01
Parity			--	--
0	−0.18 (±0.05)	0.07		
1	−0.25 (±0.06)	0.006		
2	−0.20 (±0.06)	0.01		
3+	0	--		

[a]Full models included all patient characteristics as shown in Table 1. In this table, only those patient characteristics that significantly predicted (p<.05) the independent variable are shown, if any. For Uninhibited TNF-α, there were no significantly associated patient characteristics. The only patient characteristic significantly associated with DEX IC_{50} was parity.