Cytokine 149 (2022) 155758

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Inflammatory and immune marker trajectories from pregnancy to one-year post-birth

Kharah M. Ross [a,*], Christine Dunkel Schetter [b], Judith E. Carroll [c], Roberta A. Mancuso [d], Elizabeth C. Breen [c], Michele L. Okun [e], Calvin Hobel [f], Mary Coussons-Read [e]

[a] Centre for Social Sciences, Athabasca University, Athabasca, AB, Canada
[b] Department of Psychology, University of California, Los Angeles, Los Angeles, CA, USA
[c] Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, CA, USA
[d] Department of Psychology and Neuroscience, Regis University, Denver, CO, USA
[e] University of Colorado – Colorado Springs, Colorado Springs, CO, USA
[f] Cedars-Sinai Medical Centre, Los Angeles, CA, USA

ARTICLE INFO

Keywords:
Pregnancy
Postpartum Period
Inflammatory Proteins
Parity
BMI

ABSTRACT

Background: Pregnancy is an immunomodulatory state, with reported systematic changes in inflammatory and immune activity by pregnancy stage. Published data are inconsistent as to how inflammatory and immune markers change and recover across pregnancy and the postpartum period, or the sociodemographic, health and pregnancy-related factors that could affect biomarker trajectories. The purpose of this study is to describe inflammatory and immune marker trajectories from pregnancy to a year post-birth, and to test associations with sociodemographic, health and pregnancy-related variables.

Methods: A sample of 179 pregnant women were assessed three times during pregnancy (between 8 and 36 weeks gestation) and three times during the postpartum period (between 1 and 12 months). Maternal sociodemographic characteristics, health, and pregnancy factors were obtained at study entry. Blood samples from each assessment were assayed for interleukin(IL)-6, tumor necrosis factor(TNF)α, IL-8, IL-10, and interferon(IFN)γ. Multilevel modelling was used to characterize biomarker trajectories and associations with sociodemographic and health variables.

Results: Distinct trajectories over time emerged for each biomarker. Male pregnancies were associated with higher TNFα, IL-10, and IFNγ; higher pre-pregnancy BMI was associated with higher IL-6 and IFNγ. Nulliparity was associated with greater increases in IL-6 and TNFα.

Conclusions: Patterns observed for inflammatory and immune markers from pregnancy to a year postpartum support the hypothesis that the maternal immune system changes systematically across pregnancy and through an extended postpartum period. Parity, pre-pregnancy BMI and child sex are associated with inflammatory marker patterns over time. These results contribute to our understanding of how immune system activity changes from pregnancy to the post-birth period, and the factors that could affect those changes.

1. Introduction

The immune system plays a central role in healthy pregnancy and the postpartum period. There is some evidence that maternal immune activity shifts systematically over the course of pregnancy, with early pregnancy (at the time of conception) and late pregnancy (during labor and delivery) characterized by pro-inflammatory processes, and mid-pregnancy (fetal development and growth) characterized by anti-inflammatory processes [1–3]. These changes to maternal immune activity are attributed to altered neuroendocrine signaling, as regulated by the maternal-placental-fetal unit [1], and disrupted immune activity is related to risk for adverse outcomes, such as preterm birth [4]. Altered

Abbreviations: BMI, Body Mass Index; IL, Interleukin; TNF, Tumor Necrosis Factor; IFN, Interferon; CRP, C-Reactive Protein; SES, Socioeconomic Status; LPS, Lipopolysaccharide; PBMC, Peripheral Blood Mononuclear Cell.

* Corresponding author at: Psychology, Centre for Social Sciences, Athabasca University, 1 University Dr, Athabasca, AB T9S 3A3, Canada.
E-mail address: kharahr@athabascau.ca (K.M. Ross).

https://doi.org/10.1016/j.cyto.2021.155758
Received 30 March 2021; Received in revised form 10 September 2021; Accepted 27 October 2021
Available online 11 November 2021

immune activity continues into the postpartum period (the "fourth trimester") [5], driven by recovery from labor and delivery, on-going lactation, and sleep deprivation [6]. The precise nature and timing of these pregnancy and postpartum immune adaptations, however, are still under investigation. "Normal" inflammatory patterns must be illuminated in order to identify patterns that pose risk for adverse outcomes.

Several studies have explored how peripheral immune or inflammatory proteins vary over the course of pregnancy [7–18], and into the postpartum period [19–29]. These studies are generally consistent with the observations above, i.e., that pregnancy inflammatory activity shifts systematically over time. Yet, integration of the findings has proven difficult for several reasons. First, the majority of studies – even those published within the last 5 years – have relatively small samples, by which we mean $N < 100$ or $N_{Avg} = 60$ [7,11–14,17,19–21,23,25,26,28,30]. Second, there is substantial variability concerning the number and timing of blood sampling. Although a minimum of three pregnancy assessments is more common, studies have assessed inflammatory proteins only once [22,24] or twice [8,13,15] during pregnancy. It is difficult to infer trends over time using three or fewer assessments. Third, although pregnancy-driven immune changes can persist into the postpartum period [31–33], postpartum samples are not consistently collected. If postpartum samples are collected, sampling generally occurs at 6 months postpartum or earlier, but has ranged from 3 days [19,20] to 3 years post-delivery [24]. Given that inflammatory marker concentrations may not return to pre-pregnancy levels until a year after birth [24], sample collection at 6 months postpartum or earlier may not provide an adequate understanding of postpartum inflammatory protein changes. And finally, assays with different sensitivities are used (e.g. mass cytometry, proximity extension assay multiplex, bead-based multiplex, electrochemiluminescence multiplex, and single-protein enzyme-linked immunosorbent assays), which affect ability to detect subtler patterns. In sum, small sample sizes, differences in number and timing of sampling, lack of postpartum sampling and differences in assays used make the literature difficult to evaluate or integrate.

Researchers posit that deviations from expected maternal pro- and anti-inflammatory patterns over pregnancy can pose risk for adverse pregnancy outcomes, such as preterm birth [4]. The factors that cause deviations in inflammatory protein trajectories, or the nature of those deviations, are not known. Immune activity may vary by sociodemographic characteristics. Advanced *maternal age* (>35 years) was associated with higher pro-inflammatory [interleukin (IL)-6, tumor necrosis factor (TNF)α] and immune activation [IL-2, interferon (IFN)γ] protein concentrations at 8.5 and 25 weeks gestation in a sample of 1,274 Danish women [8]. *Race/ethnicity* could also be associated with inflammation during pregnancy. Being a member of a racial/ethnic minority is associated with increased risk for adverse pregnancy outcomes, especially preterm birth and preeclampsia [34], and possibly higher pro-inflammatory markers during pregnancy (e.g. IL-6) in part due to chronic stress, such as discrimination exposure [35]. One study tested whether race/ethnicity was associated with changes in inflammatory protein production following peripheral blood mononuclear cell activation with lipopolysaccharide (LPS) in a sample of Black and White pregnant women [36]. No systematic differences by race were observed, but it is also not clear how inflammatory protein production following immune activation relates to levels of peripheral inflammatory markers. Finally, lower *socioeconomic status* (SES) is associated with a number of inflammatory outcomes during pregnancy, including evidence of chronic inflammation in the placenta at birth [37,38] and higher peripheral IL-6 and C-reactive protein (CRP), as mediated by pre-pregnancy body mass index (BMI) [39]. Although maternal age, race/ethnicity, and SES may be associated with inflammatory activity from pregnancy to the postpartum period, no studies have directly tested associations between these sociodemographic characteristics and inflammatory protein trajectories.

Other important health and perinatal risk factors could affect inflammatory protein trajectories over pregnancy, such as parity, maternal pre-pregnancy BMI, and fetal sex. Being parous, or having had a previous birth, is associated with persistent changes in immune activity. For example, T-regulatory cells that migrate into the decidua, or mucosal lining of the uterus, persist after delivery and rapidly accumulate during subsequent pregnancies [1], suggesting what is referred to as "pregnancy memory" [40]. One study tested whether parity was associated with inflammatory protein levels at 8.5 and 25 weeks gestation in a sample of 1,274 Danish women but found no associations [8]. Again, inflammatory protein trajectories were not modeled.

Pre-pregnancy BMI is also associated with inflammatory outcomes during pregnancy. Adipose tissue produces pro-inflammatory cytokines, e.g. IL-6 [41], and so higher BMI could affect immune system regulation over pregnancy. Higher pre-pregnancy BMI is associated with immune or inflammatory protein concentrations over pregnancy, but specific patterns vary. For example, in two studies, higher pre-pregnancy BMI was associated with higher pro-inflammatory markers (e.g. IL-6 and TNFα) at time points between 11 weeks gestation and 4 months postpartum [23,42]. Other studies reported that higher pre-pregnancy BMI was not associated with IL-6 or TNFα, but was associated with higher IFNγ at 8.5 and 25 weeks gestation [8], and that it was not associated with IL-6 at 10 to 14 and 26 to 28 weeks gestation, preceding delivery, and 3 days postpartum [19]. Fetal sex is sometimes implicated in maternal immune activity over pregnancy. In a study of 38 pregnancies, carrying a male fetus was associated with higher levels of some pro-inflammatory proteins (e.g. IL-12p70, IL-21) at multiple time points over pregnancy [43], whereas in another sample of 80 pregnant women, fetal sex was not associated with inflammatory protein production following peripheral blood mononuclear cell (PBMC) activation with LPS over pregnancy, but not peripheral inflammatory markers over pregnancy [12]. Finally, infection during pregnancy increases risk for adverse outcomes, such as preterm birth [44]. Whether infections are associated with altered inflammatory trajectories over pregnancy and the postpartum period is not known.

The primary purpose of this study was to document peripheral blood concentrations of immune and inflammatory proteins (IFNγ, IL-10, IL-6, IL-8, TNFα) at multiple time points from pregnancy (8 to 16 weeks gestation) to one-year post-birth. Further, we hypothesized that factors previously associated with inflammatory levels during pregnancy and the postpartum period, namely sociodemographic characteristics (maternal age, race/ethnicity, SES) and/or health and perinatal risk factors (parity, pre-pregnancy BMI, fetal sex, infections), could be related to trajectories of immune and inflammatory proteins during and following pregnancy.

2. Methods

2.1. Participants

The sample consisted of 179 pregnant women recruited from Los Angeles, CA, and Denver, CO, as part of the Healthy Babies Before Birth (HB3) study. Inclusion criteria were 18 years of age or older and singleton pregnancies of up to 12 weeks gestation at time of recruitment. Exclusion criteria were current substance abuse, HIV-positive status, current smoking, or medications that could affect inflammatory processes, such as glucocorticoids, either at the time of recruitment or while participating. A sample of 294 women enrolled in the study and completed baseline measures. Of those, 199 participants had inflammatory marker data available at two or more time points. Complete data on all variables were only available for 179 participants [20 participants were excluded due to missing data for gestational age at study entry (33%), maternal age (21%), marital status (21%), SES (29%), race/

ethnicity (21%) and child sex (21%)]. All participants provided informed consent, and protocols and procedures were reviewed and approved by IRBs at the University of California – Los Angeles, University of Colorado – Colorado Springs, Denver University and Cedars-Sinai Medical Centre in Los Angeles.

2.2. Procedure

Women completed interviews and provided blood samples at three time points during pregnancy (at study entry at 8–16 weeks gestation [T1], 20–26 weeks gestation [T2], and 30–36 weeks gestation [T3]), and at three time points during the postpartum period (1 month [P1], 6 months [P2], and 12 months [P3] postpartum) in prenatal settings and healthcare or university labs after birth. Medical variables on birth outcomes and pregnancy risks were also abstracted from medical charts.

2.3. Sociodemographic variables

At study entry, women provided information on sociodemographic characteristics: (1) Age (at study entry, years), (2) self-identified race/ethnicity, (3) socioeconomic status (SES) based on education and household income, and (4) marital status (married or cohabiting or not). Race/ethnicity was dummy coded using two variables: "Race Hispanic" was coded as Hispanic (1) and White or Other race (0), and "Race Other" was coded as Other Race (1) and Hispanic or White (0). No participants identified as Black Hispanic.

SES was computed by standardizing education and adjusted per capita household income and taking the average of the standardized scores. Higher values indicate higher SES. Adjusted per capita household income was calculated by dividing household income by the number of individuals in the household.

2.4. Pregnancy and health variables

Pregnancy and health-related variables were parity (nulliparous or not), child sex (0 = male or 1 = female), and evidence of any infection during pregnancy. Number of prior live births (parity) was reported by mothers at study entry and was also in medical charts. Evidence of any infections during pregnancy was based on medical chart abstraction by trained staff and maternal self-report, and could include sexually transmitted infections, urinary tract infections, vaginal infections or other infections. Women were coded as yes (1) or no (0) regarding the presence of any infections during pregnancy. Gestational age at blood sample collection was calculated by subtracting conception date as determined through ultrasound from the assessment date. Gestational age at study entry (T1) was included as a covariate to equate all participants with respect to gestation at baseline.

At study entry, participants reported their last pre-pregnancy weight. Maternal height was measured by study personnel at baseline using a balance-beam scale. Pre-pregnancy BMI (kg/m^2) was calculated by taking the last pre-pregnancy weight (kg) and dividing by height squared (m^2).

Preterm birth (less than 37 weeks gestation), low birth weight (less than 2500 g), and obstetric risk were available and also considered as predictors. Obstetric risk was calculated as per previous research [44], defined as presence of historical risk factors and medical conditions that increase risk for preterm birth, including previous history of preterm birth, vaginal bleeding, hypertension, diabetes and evidence of infection. Obstetric risk was coded as 'any' (1) and 'none' (0). Preterm birth, low birth weight and obstetric risk were not associated with inflammatory protein outcomes (p's > 0.05), and were excluded from final models.

2.5. Maternal plasma inflammatory protein concentrations

Blood samples were obtained by venipuncture into an EDTA Vacutainer tube (BD Bioscience). If participants reported an active infection at the time of assessment, the blood draw was rescheduled to a time after the infection had resolved. Blood samples were processed to plasma immediately post-collection, and stored at −80C until assay. Cytokines were measured by electrochemiluminescent immunoassay on a MesoScale Discovery (MSD) MESO QuickPlex SQ 120 instrument and Discovery Workbench software, using an MSD V-PLEX Custom Human Cytokine Proinflammatory Panel [IFNγ, IL-10, IL-6, IL-8 and TNFα] (MSD, Rockville, MD). The five additional analytes available on the full panel (IL-1β, IL-2, IL-4, IL-12p70, and IL-13) were not utilized based on pilot studies with HB3 pregnancy samples that indicated the majority of samples were below the limit of detection for these analytes (data not shown). MSD assays were performed in duplicate at a 2-fold sample dilution according to the manufacturer's protocol, with an extended standard curve at the lower end that utilized an 8-point curve of serial 3-fold dilutions, beginning with a 3-fold dilution of the multianalyte calibrator provided by the manufacturer. All samples from a single participant were assayed on the same plate, and all plates were from the same kit lot; an internal quality control sample was included on every plate to monitor assay reproducibility. Analyte-specific lower limits were calculated for each assay plate, with typical lower limits of 0.1 pg/mL for IL-10, 0.2–0.3 pg/mL for IL-6 and TNFα, and 0.6 pg/mL or less for IFNγ and IL-8. Samples with concentrations below the lower limit of detection for a given analyte (2.9% of samples for IL-10, 3.0% for IL-6, 0.5% for IL-8) were assigned values equal to the plate-specific lower limit of detection (LLD)/$\sqrt{2}$ [9]. Inter-assay coefficients of variation were less than 10% for IL-6 and IL-8, less than 12% for IFNγ and TNFα, and 16% for IL-10 (which was expected due to very low concentrations). Mean intra-assay coefficients of variation were less than 10% for all analytes.

2.6. Analytic strategy

Cytokine concentration distributions were positively skewed and were natural log (ln) transformed prior to analysis. Two-level multilevel models were used to determine cytokine trajectories over the follow-up (pregnancy to a year post-birth), and associations between sociodemographic, pregnancy, and health variables and cytokine protein trajectories, as shown in Equation 1. All models were run using HLM 8.00 [45]. Estimated trajectories and intercepts were allowed to vary randomly. Models were estimated with robust standard errors and restricted maximum likelihood (REML). Within-person variables modeling time (months) were entered uncentered at Level 1. Linear (Time), quadratic ($Time^2$), and cubic ($Time^3$) trends in trajectories were modeled. Between-person variables (sociodemographic, pregnancy, and health variables) were grand centered and entered at Level 2. Cytokine trajectories were calculated by subbing appropriate values into the multilevel model regression equations [46,p. 63–65]

Equation 1 models both the intercept (π_{0i}) and trajectories over time ($\pi_{1i} - \pi_{3i}$). Within the intercept equations, the coefficient β_{00} can be interpreted as the average, adjusted cytokine protein concentration at study entry. The remaining coefficients (β_{0i}) indicate associations between other variables and the intercept or cytokine protein concentrations at study entry. Within the trajectory equations ($\pi_{1i} - \pi_{3i}$), the coefficients β_{10}, β_{20} and β_{30} indicate the average, adjusted within-person changes or trajectories over the follow-up (linear, quadratic and cubic influences, respectively). The remaining coefficients are cross-level interaction terms (between Level 2 variables and Level 1 linear, quadratic or cubic trends in time). Cross-level interactions were probed by calculating cytokine trajectories at different levels of the Level 2 variable. For continuous variables, trajectories were calculated at the mean and +/- 1 SD. For dichotomous variables, trajectories were calculated for each coded value.

Equation 1:

$$[Cytokine]_{ti} = \pi_{0i} + \pi_{1i}*(Time) + \pi_{2i}*(Time^2) + \pi_{3i}*(Time^3) + e_{ti}$$

$$\pi_{0i} = \beta_{00} + \beta_{01}*(PreBMI) + \beta_{02}*(EntryGA) + \beta_{03}*(Age) + \beta_{04}*(Marital\ Status) + \beta_{05}*(SES) + \beta_{06}*(Race\ Hispanic) + \beta_{07}*(Race\ Other) + \beta_{08}*(Parity) + \beta_{09}*(Child\ Sex) + \beta_{10}*(Infection) + r_{0i}$$

$$\pi_{1i} = \beta_{10} + \beta_{11}*(PreBMI) + \beta_{12}*(Entry\ GA) + \beta_{13}*(Age) + \beta_{14}*(Marital\ Status) + \beta_{15}*(SES) + \beta_{16}*(Race\ Hispanic) + \beta_{17}*(Race\ Other) + \beta_{18}*(Parity) + \beta_{19}*(Child\ Sex) + \beta_{110}*(Infection) + r_{0i}$$

$$\pi_{2i} = \beta_{20} + \beta_{21}*(PreBMI) + \beta_{22}*(Entry\ GA) + \beta_{23}*(Age) + \beta_{24}*(Marital\ Status) + \beta_{25}*(SES) + \beta_{26}*(Race\ Hispanic) + \beta_{27}*(Race\ Other) + \beta_{28}*(Parity) + \beta_{29}*(ChildSex) + \beta_{210}*(Infection) + r_{0i}$$

$$\pi_{3i} = \beta_{30} + \beta_{31}*(PreBMI) + \beta_{32}*(EntryGA) + \beta_{33}*(Age) + \beta_{34}*(Marital\ Status) + \beta_{35}*(SES) + \beta_{36}*(Race\ Hispanic) + \beta_{37}*(Race\ Other) + \beta_{38}*(Parity) + \beta_{39}*(Child\ Sex) + \beta_{310}*(Infection) + r_{0i}$$

3. Results

Sample characteristics are presented in Table 1, and correlations in Table S1. Participants were on average 31 +/- 5.8 years old at study entry, primarily non-Hispanic White (46%) or Hispanic (36%), and roughly half nulliparous (57%). The sample was also evenly divided with respect to child sex (46% female; 54% male).

Coefficients for multilevel models predicting covariate-adjusted immune and inflammatory protein levels at study entry (T1) and trajectories from pregnancy to one-year post-birth appear in Table S2. All figures show adjusted immune protein trajectories. All reported associations between a predictor and inflammatory protein intercepts or trajectories are independent of all other predictors.

3.1. Il-6

IL-6 is a pro-inflammatory cytokine, involved in activating or up-regulating inflammatory responses [47,48]. There was a significant linear, $\beta_{10} = 0.027$, $SE = 0.008$, $p = .001$, and quadratic, $\beta_{20} = -0.071$, $SE = 0.001$, $p = .040$, trend in IL-6 trajectories from pregnancy to a year post-birth. Specifically, IL-6 gradually increased from study entry (8 to 16 weeks gestation) to labor and delivery, then, it gradually decreased over the post-birth period (Fig. 1).

Pre-pregnancy BMI, SES, and parity were associated with IL-6 from pregnancy to a year post-birth. Higher pre-pregnancy BMI was associated with higher IL-6 at study entry, $\beta_{01} = 0.018$, $SE = 0.004$, $p < .001$, but was not associated with differences in trajectories from pregnancy to a year post-birth, p's > 0.269. SES was not associated with IL-6 at study entry, $\beta_{05} = -0.002$, $SE = 0.026$, $p = .939$, but did interact with linear trends in IL-6 over time, $\beta_{15} = -0.022$, $SE = 0.011$, $p = .047$. Specifically, lower SES was associated with steeper increases in IL-6 over the pregnancy period (Fig. 2).

Finally, although parity was not associated with IL-6 at study entry, $\beta_{08} = 0.008$, $SE = 0.051$, $p = .869$, parity interacted with linear, $\beta_{18} = -0.050$, $SE = 0.021$, $p = .020$, quadratic, $\beta_{28} = 0.007$, $SE = 0.003$, $p = .012$, and cubic, $\beta_{38} = -2.4\text{x}10^{-4}$, $SE = 9.5\text{x}10^{-5}$, $p = .012$, trends in IL-6 over time. As shown in Fig. 3A, nulliparous pregnancies were characterized by quadratic trends in IL-6 over time, with increasing IL-6 from pregnancy to labor and delivery, followed by gradual decreases over the post-birth period. In contrast, parous pregnancies were characterized by cubic trends in IL-6 over time, characterized by lower IL-6 during pregnancy, followed by increasing IL-6 to 6 months post-birth, and decreases to 12 months post-birth.

Table 1
Sample characteristics (N = 179).

Variable	Mean +/- SD or % (N)
Site (Los Angeles)	61% (109)
Age (years)	31.0 +/- 5.8
Race/ethnicity Non-Hispanic White	44% (80)
Hispanic	35% (64)
Black	9% (16)
Asian	8% (14)
Multi-race	3% (5)
Per capita household income ($)	25,554 +/- 24,391
Education (years)	15.9 +/- 3.2
Marital status (married)	74% (132)
Gestational age at study entry (weeks)	14.0 +/- 1.7
Gestational age at birth (weeks)	39.3 +/- 2.1
Pre-pregnancy BMI (kg/m2)	26.3 +/- 7.1
Nulliparous	56% (100)
Child sex (female)	45% (81)
Any infection during pregnancy	51% (93)
Time of assessment after study entry T1	0.0 +/- 0.0 months (14.0 weeks gestation)
T2	1.9 +/- 0.5 months (21.6 weeks gestation)
T3	4.3 +/- 0.6 months (31.2 weeks gestation)
P1	7.5 +/- 0.6 months (1.18 months postpartum)
P2	12.1 +/- 0.6 months (5.78 months postpartum)
P3	18.2 +/- 0.7 months (11.9 months postpartum)

3.2. TNFα

TNFα is another pro-inflammatory cytokine, involved in activating or up-regulating inflammatory responses [49]. There was a significant linear, $\beta_{10} = 0.020$, $SE = 0.005$, $p < .001$, and cubic, $\beta_{30} = -7.60\text{x}10^{-5}$, $SE = 3.2\text{x}10^{-5}$, $p = .018$, trend in TNFα trajectories from pregnancy to a year post-birth. TNFα increased from study entry (8 to 16 weeks gestation) to 6 months post-birth, then decreased slightly to a year post-birth (Fig. 1).

Child sex was associated with TNFα at study entry, $\beta_{09} = -0.068$, $SE = 0.029$, $p = .023$, such that being pregnant with a male fetus was associated with higher TNFα. Although parity was not associated with TNFα at study entry, $\beta_{08} = 0.065$, $SE = 0.034$, $p = .057$, parity interacted with linear, $\beta_{18} = -0.039$, $SE = 0.011$, $p < .001$, quadratic, $\beta_{28} = 0.006$, $SE = 0.002$, $p = .003$, and cubic, $\beta_{38} = -2.03\text{x}10^{-4}$, $SE = 7.20\text{x}10^{-5}$, $p = .006$, trends in TNFα over time. As shown in Fig. 3B, nulliparity was associated with cubic trends in TNFα, such that TNFα began to increase later in pregnancy, continued to increase to 6 months post-birth, and then decreased to 12 months post-birth. In contrast, parity was associated with earlier increases in TNFα during pregnancy, but smaller overall increases in TNFα to 6 months post-birth, followed by similar decreases in TNFα to 12 months post-birth.

3.3. IL-8

IL-8 (also known as CXCL8) is a chemokine involved in neutrophil recruitment, either in an inflammatory response to an immune challenge or to facilitate tissue remodeling [50]. There was a significant quadratic, $\beta_{20} = 0.010$, $SE = 0.002$, $p < .001$, and cubic, $\beta_{30} = -4.48\text{x}10^{-4}$, $SE = 8.1\text{x}10^{-5}$, $p < .001$, trend in IL-8 trajectories from pregnancy to a year post-birth. Specifically, IL-8 increased from study entry (8 to 16 weeks gestation) through to 6 months post-birth, and then decreased slightly to 12 months post-birth (Fig. 1).

Age and race/ethnicity were associated with IL-8 at study entry. Specifically, older age, $\beta_{03} = 0.010$, $SE = 0.005$, $p = .041$, and being of Hispanic ethnicity, $\beta_{06} = 0.110$, $SE = 0.047$, $p = .020$, were associated with higher IL-8 at study entry. Neither age nor race/ethnicity, however, were associated with IL-8 trajectories from pregnancy to a year post-birth, p's > 0.684.

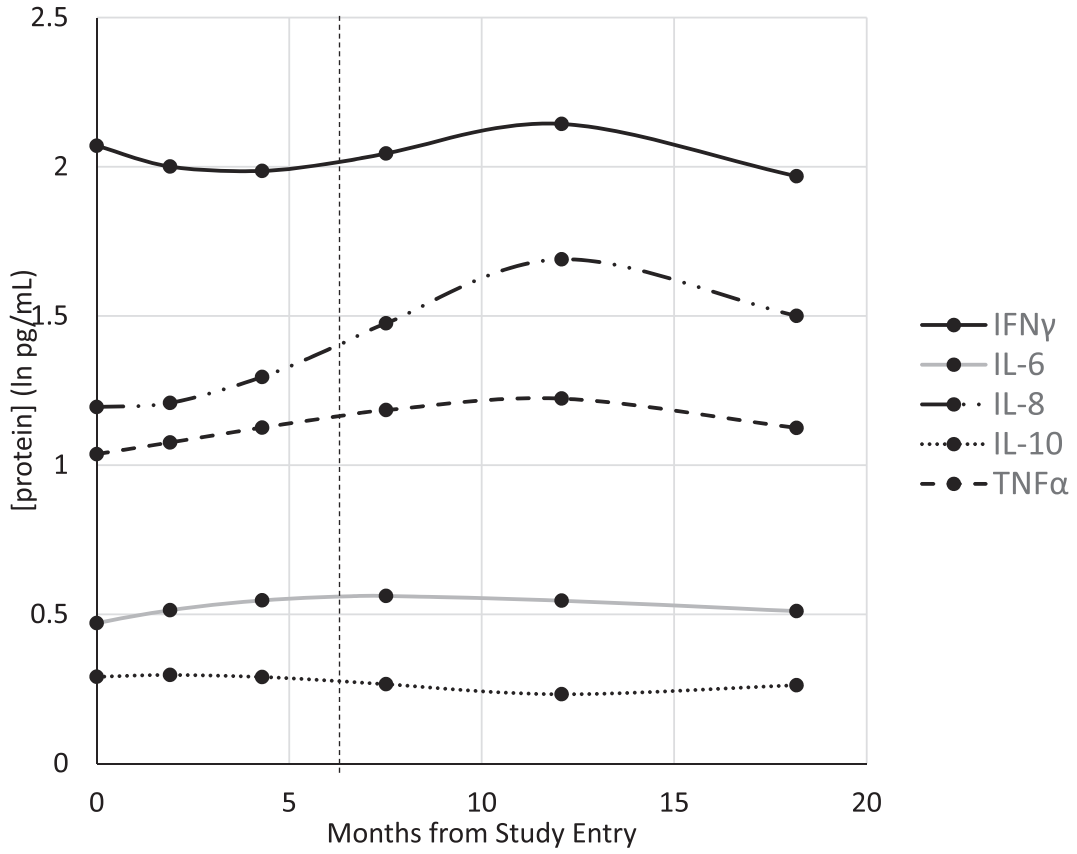

Fig. 1. Adjusted protein marker trajectories from study entry (8–16 weeks gestation) to a year post-birth. The vertical, dashed line represents average timing of labor and delivery.

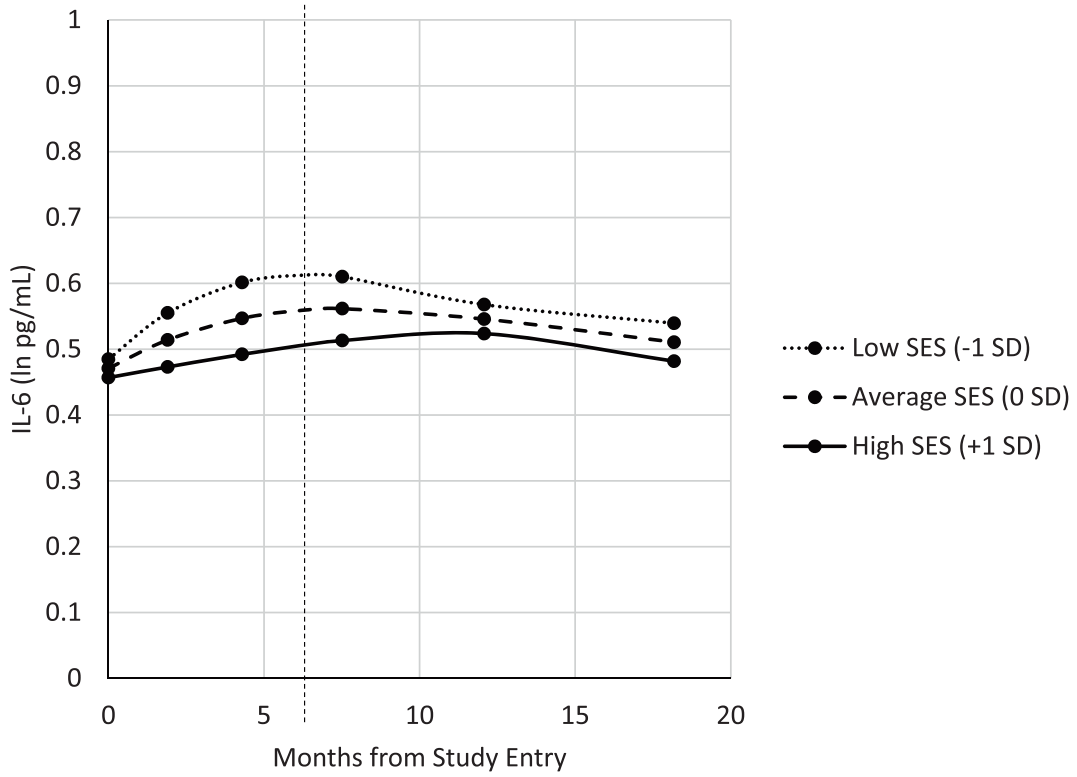

Fig. 2. Adjusted IL-6 trajectories from study entry (8–16 weeks gestation) to one-year post-birth by maternal SES. The vertical, dashed line represents average timing of labor and delivery.

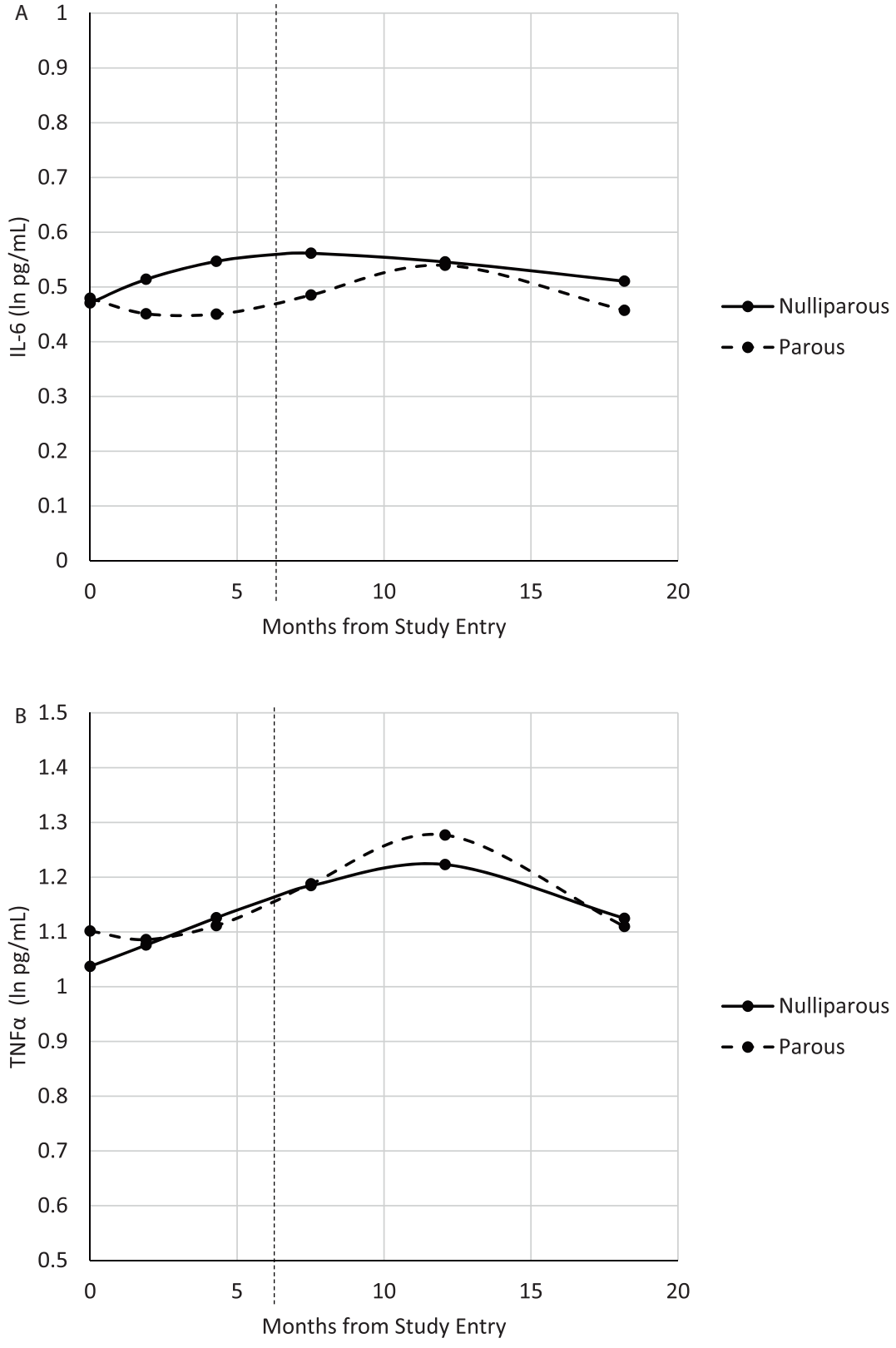

Fig. 3. A. Adjusted IL-6 trajectories from study entry (8–16 weeks gestation) to one-year post-birth by parity. The vertical, dashed line represents average timing of labor and delivery. B. Adjusted TNFα trajectories from study entry (8 to 16 weeks gestation) to one-year post-birth by parity. The vertical, dashed line represents average timing of labor and delivery.

3.4. IL-10

IL-10 is an anti-inflammatory cytokine, involved in regulating (or down-regulating) inflammatory responses [51–54]. There was a marginally significant quadratic, $\beta_{20} = -0.002$, $SE = 0.001$, $p = .097$, and cubic trend, $\beta_{30} = 0.8.2x\ 10^{-4}$, $SE = 4.8x10^{-4}$, $p = .086$, in IL-10 over time. As shown in Fig. 1, IL-10 decreased slightly from study entry (8 to 16 weeks gestation) to 6 months after-birth, followed by a slight increase to 12 months post-birth. Of note, IL-10 had lowest total variability ($\sigma^2_{Total} = 0.020$), which reduced power to detect effects.

Of the sociodemographic, pregnancy and health factors considered, only child sex was associated with IL-10 at study entry, $\beta_{09} = -0.071$, $SE = 0.025$, $p = .004$. Specifically, being pregnant with a male fetus was associated with higher IL-10 at pregnancy.

3.5. IfNγ

IFNγ is secreted by natural killer (NK) cells and Th1 cells, and is involved in regulating innate and adaptive viral immunity [55]. There were significant linear, $\beta_{10} = -0.053$, $SE = 0.024$, $p = .027$, quadratic, $\beta_{20} = 0.009$, $SE = 0.004$, $p = .019$, and cubic, $\beta_{30} = -3.76x10^{-4}$, $SE = 1.59x10^{-4}$, $p = .019$, trends in IFNγ trajectories over time (Fig. 1). IFNγ demonstrated strong cubic trends, with a general decrease from pregnancy to late-pregnancy, an increase from late-pregnancy to 6 months after-birth, followed by a decrease from 6 to 12 months after birth.

Pre-pregnancy BMI and Race-Other (i.e. not White or Hispanic) were associated with IFNγ at study entry only. Specifically, higher pre-pregnancy BMI, $\beta_{01} = 0.022$, $SE = 0.009$, $p = .011$, and not being Hispanic or non-Hispanic White, $\beta_{06} = 0.265$, $SE = 0.115$, $p = .023$, were associated with higher IFNγ at study entry.

Child sex was also associated with IFNγ at study entry, $\beta_{09} = 0.022$, $SE = 0.009$, $p = .011$, such that being pregnant with a male fetus was associated with higher IFNγ at study entry. Child sex also interacted with linear trends in IFNγ over time, $\beta_{19} = 0.089$, $SE = 0.045$, $p = .048$, but not quadratic or cubic trends in time, p's > 0.136. As shown in Fig. 4, being pregnant with a female fetus is associated with steeper increases in IFNγ slope, on average, from pregnancy to a year post-birth, compared to being pregnant with a male fetus.

4. Discussion

The purpose of this study was to characterize inflammatory and immune protein trajectories (IL-6, TNFα, IL-8, IL-10, and INFγ) at six times from early pregnancy (8–16 weeks) to one-year after birth, and test whether sociodemographic characteristics, pregnancy, and health factors were associated with those trajectories over time. Multilevel models illustrate that there are differences in the trajectories of inflammatory and immune proteins over this period, with some proteins demonstrating little or no change (IL-10) to proteins demonstrating substantial fluctuations (IL-8, INFγ) from pregnancy to a year post-birth. Furthermore, although several factors were independently associated with inflammatory and immune protein concentrations over this period (notably parity, child sex, and pre-pregnancy BMI), specific patterns varied by protein and sociodemographic, prenatal, or health variables considered, as described below. In sum, these findings highlight the complexity of pregnancy and postpartum immune and inflammatory adaptations, and how sociodemographic, pregnancy, or health factors may affect those adaptations.

Pregnancy is characterized as an immunomodulatory state, with specific inflammatory activity depending on pregnancy stage or fetal development [1]. Earlier (conception) and later (labor and delivery) pregnancy are characterized by extensive tissue remodeling, and are thus expected to be pro-inflammatory states [1]. In contrast, mid-pregnancy is characterized by fetal growth and development, and a shift towards an anti-inflammatory or Th2 dominance. Postpartum processes are also characterized by tissue remodeling (lactation), wound healing or recovery from pregnancy and labor and delivery, and sleep deprivation [22,24], and so is also expected to be a pro-inflammatory

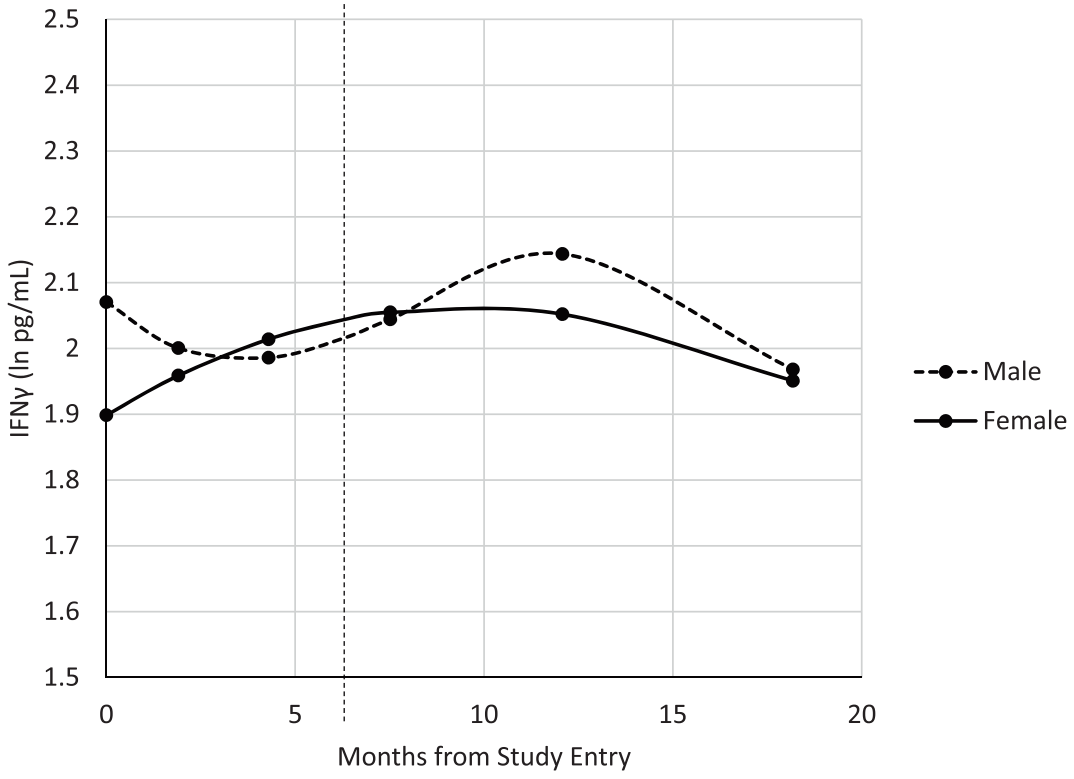

Fig. 4. Adjusted IFNγ trajectories from study entry (8 to 16 weeks gestation) to one-year post-birth by child sex. The vertical, dashed line represents average timing of labor and delivery.

state. In general, this pattern is consistent with our findings and those of others. Increases in pro-inflammatory cytokines, IL-6 and TNFα, were observed from study entry (8 to 16 weeks gestation) to 6 months postpartum, followed by decreases to a year post-birth, which is consistent with many other studies [10,15,22–25,36] but not all[c.f.,25,56] . Although not statistically significant at $p < .05$, a complementary but opposite trend was observed for anti-inflammatory IL-10, with slight decreases from pregnancy to 6-months postpartum, followed by a slight increase to a year post-birth. Again, this is consistent with previous research [15,19,24] increasing confidence in the result. Observed changes in IL-8, a chemokine that recruits neutrophils to tissue to facilitate inflammatory immune responses and tissue remodeling, are also consistent with the immunomodulatory model of pregnancy and postpartum tissue remodeling and post-labor and delivery recovery [22,24]. Collectively, this pattern is consistent with the immunomodulatory model of pregnancy, with shifts from anti-inflammatory preponderance mid-pregnancy to pro-inflammatory states through late pregnancy and 6-months postpartum.

Finally, IFNγ, a cytokine secreted by natural killer (NK) cells and Th1 cells involved in regulating innate and adaptive viral immunity, decreased from study entry (8 to 16 weeks gestation) to late-pregnancy, followed by increases up to 6 months postpartum, and another decrease by one year post-birth. These trajectories are consistent with other studies [15,25,26], but it is not clear if this pattern is consistent with the immunomodulatory model of pregnancy. Although some researchers propose that increases in IFNγ mid-pregnancy indicates an up-regulation of innate viral immunity to counter or compensate for down-regulation of adaptive or cellular-mediate viral immunity (Th1) [25], IFNγ also plays a role in blocking Th2 cell activity [55], or the branch of the adaptive immune system that counter-regulates Th1 activity. Increased concentration of an anti-Th2 cytokine mid-pregnancy may not be consistent with the immunomodulatory model hypothesis that mid-pregnancy is a predominately Th2 state. The immunomodulatory model literature has not extensively considered IFNγ, focusing instead on Type 1 interferons (e.g. IFNα or IFNβ) [1]. Nonetheless, additional research is necessary to understand the role of IFNγ during pregnancy, and whether this is consistent with the immunomodulatory model.

Collectively, this pattern of findings highlights some important issues in the current literature. First, it has been suggested that the immunomodulatory phases of pregnancy occur during discrete periods with, for example, the Th2-dominant or anti-inflammatory period of pregnancy occurring between 13 and 27 weeks gestation [1]. This window is not necessarily supported by this or other research, which detected increases in pro-inflammatory cytokines as early as 14 and 22 weeks gestation. The various immunomodulatory phases of pregnancy might not occur within specific windows, but instead could develop or shift gradually and accumulate over pregnancy.

Second, methodological differences in timing, number of samplings, and type of immune tissue accessed could affect the pattern detected. Studies should collect at least three or more samples during pregnancy, in order to capture the complex changes in inflammatory proteins. Indeed, results from the LIFECODES birth cohort suggest that TNFα could fluctuate several times over pregnancy, rather than steadily increase [9,10]. And given that pregnancy-related changes in inflammatory markers can persist up to 6 months postpartum, samples collected at 6 months postpartum or earlier should not be treated as a normative or non-pregnancy comparison. Indeed, differences in postpartum sample timing may be why IL-8 trajectories were described as U-shaped, as opposite to fluctuating increases and decreases, in other studies [19,23]. Studies of spontaneous cytokine production by PBMCs are not comparable to studies of peripheral cytokine concentrations, which also include inflammatory proteins produced by non-immune tissues, such as the placenta [57,58].

Third, this body of research suggests that care is needed when inferring how pregnancy inflammatory markers are expected to relate to adverse pregnancy outcomes. Researchers tend to frame "pro-inflammatory" activity during pregnancy as conferring increased risk for adverse outcomes, such as preterm birth [1]. This and other research suggests, however, that pregnancy stage at the time of sampling could affect whether pro-inflammatory protein concentrations are considered normal or not [1].

In addition to presenting levels over time and trajectories in immune markers, the role of sociodemographic, pregnancy, and health factors with respect to inflammatory protein trajectories was examined. Child sex, pre-pregnancy BMI, and parity were consistently associated with inflammatory protein levels and trajectories from pregnancy to one year post-birth. Male sex was associated with higher concentrations of anti-inflammatory IL-10 and pro-inflammatory TNFα at baseline and change from pregnancy to a year post-birth. Male sex was also associated with higher IFNγ in pregnancy, and overall steeper increases in IFNγ from pregnancy to a year post-birth, compared to female sex. This is consistent with a previous study that reported increased pro-inflammatory cytokine levels in male pregnancies compared to female pregnancies [43], possibly driven by stronger maternal immune responses to male fetuses.

Also, higher maternal pre-pregnancy BMI was associated with higher concentrations of IFNγ and IL-6 at study entry and change from pregnancy to a year post-birth. This is also consistent with previous research [8,19,23,42], and possibly reflects a consistent pro-inflammatory influence of having more adipose tissue across pregnancy and the postpartum period. Parity was also associated with similar pro-inflammatory IL-6 and TNFα trajectories. Specifically, nulliparous women demonstrated earlier and consistent increases in IL-6 and TNFα from mid-to late-pregnancy compared to parous women. This is consistent with previous research indicating immunological "pregnancy memory" [1,40], and suggests that a nulliparous pregnancy could be immunologically different from subsequent pregnancies.

Age, race/ethnicity and SES were also associated with inflammatory markers and their trajectories, although a less consistent pattern emerged across the inflammatory markers. Older age was associated with higher IL-8 between study entry (8 to 16 weeks gestation) and a year post-birth. Although this is consistent with an association between older age and pro-inflammatory phenotype in a previous study [8], the specific inflammatory markers associated with age differed between this and that study. Differences by race/ethnicity were observed here. Not being Hispanic or non-Hispanic White was associated with higher IFNγ between pregnancy and a year post-birth, and being Hispanic was associated with higher IL-8 between pregnancy and a year post-birth. The association between race/ethnicity and pregnancy and postpartum inflammatory marker trajectories has not been well-explored, and although these findings suggest racial/ethnic differences in systemic inflammatory markers could occur, additional research is necessary to understand these patterns and their implications. Finally, lower SES was associated with steeper increases in pro-inflammatory IL-6 between pregnancy and a year post-birth, which is also consistent with previous studies [37–39]. Of interest, although half the sample reported having an infection during pregnancy, infection status was not associated with inflammatory protein levels or trajectories. It is possible that infections produce acute "spikes" in inflammatory protein levels that might not be captured in trajectories over time. It is also possible that no effect was detected because our data were not able to differentiate between acute or chronic infections, moderate or severe infections, or differences in infection onset or infection resolution. Future research should test whether these nuanced aspects of infection are associated with inflammatory protein trajectories. In sum, several sociodemographic, pregnancy, and health variables – particularly child sex, parity, pre-pregnancy BMI - are associated with inflammatory protein concentrations during pregnancy and between pregnancy and a year post-birth, but the specific patterns varied depending on inflammatory protein. Again, this speaks to the complexity of immunological adaptations during pregnancy, and how other factors could affect those adaptations.

A strength of these findings is the longitudinal design with a

sufficient sample size at all time points to test the research questions. In addition, the sample is composed of women from two health care settings and they are not uniformly middle class or White in race/ethnicity. However, although these findings are generally consistent with previous research, this sample was not recruited to be representative of the US population, and it is possible that inflammatory protein trajectories could vary depending on sample composition, particularly with respect to participant sociodemographic characteristics, such as race/ethnicity or SES, which were associated with inflammatory protein trajectories here. In addition, no study was found that examined prenatal and postpartum inflammatory protein trajectories in participants from a non-Western country, and it is not known whether these patterns represent global patterns. Another limitation is that, although these findings reflect systematic changes in peripheral inflammatory marker concentrations over pregnancy and through one year after birth, they cannot be generalized to other immune tissues (e.g. PBMCs, maternal-fetal interface).

In conclusion, inflammatory and immune proteins demonstrate changes in peripheral concentrations from pregnancy to a year post-birth, and the nature of these changes varies by which inflammatory protein is considered. Overall, these trajectories in inflammatory protein are consistent with the immunomodulatory model of pregnancy. Several variables, including parity, pre-pregnancy BMI, and child sex, are associated with differences in inflammatory and/or immune protein levels and trajectories, and patterns differ by which variable and protein are considered. These findings highlight the complex, systemic immunological adaptations that occur during pregnancy and the postpartum period, and suggest that closer examination of the interaction between a wide variety of variables and immune markers is warranted to develop a complete picture of the role of the immune system in pregnancy and birth.

CRediT authorship contribution statement

Kharah M. Ross: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **: . Christine Dunkel Schetter:** Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. **Judith E. Carroll:** Methodology, Resources, Writing – review & editing. **Roberta A. Mancuso:** Project administration, Methodology, Writing – review & editing. **Elizabeth C. Breen:** Methodology, Resources, Writing – review & editing. **Michele L. Okun:** Methodology, Writing – review & editing. **Calvin Hobel:** Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. **Mary Coussons-Read:** Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Healthy Babies Before Birth (HB3) Study was supported by funding from NIH (simple) (R01 HD073491: MPI Coussons-Read & Dunkel Schetter). REDCap is supported by the Colorado Clinical & Translational Sciences Institute (CCTSI) with the Development and Informatics Service Center (DISC) grant support [NIH/NCRR Colorado CTSI Grant Number UL1 RR025780]. The authors thank the staff and the participants, especially Susan Jackman, RN, coordinator of the Cedars Sinai Health Center site.

All authors contributed to the manuscript and consented to have their names included. Ross was responsible for the manuscript framing, analyses and writing. Dunkel Schetter and Coussons-Read were PIs on the Health Baby Before Birth Study. Carroll and Breen were instrumental in biomarker assays and interpretation. Mancuso and Hobel were involved in data collection and project coordination. All co-authors provided critical review of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2021.155758.

References

[1] G. Mor, P. Aldo, A.B. Alvero, The unique immunological and microbial aspects of pregnancy, Nat. Rev. Immunol. 17 (8) (2017) 469–482.
[2] G. Mor, I. Cardenas, The immune system in pregnancy: a unique complexity, Am. J. Reprod. Immunol. 63 (6) (2010) 425–433.
[3] G. Mor, et al., Inflammation and pregnancy: the role of the immune system at the implantation site, Ann. N. Y. Acad. Sci. 1221 (2011) 80–87.
[4] M. Cappelletti, et al., Inflammation and preterm birth, J. Leukoc. Biol. 99 (1) (2016) 67–78.
[5] H.L. Paladine, C.E. Blenning, Y. Strangas, Postpartum Care: An Approach to the Fourth Trimester, Am. Fam. Physician 100 (8) (2019) 485–491.
[6] Cunningham, G.F., et al., *Williams Obstetrics*. 2014, McGraw Hill Professional.
[7] M.E. Coussons-Read, M.L. Okun, C.D. Nettles, Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy, Brain Behav. Immun. 21 (3) (2007) 343–350.
[8] A.E. Curry, et al., Maternal plasma cytokines in early- and mid-gestation of normal human pregnancy and their association with maternal factors, J. Reprod. Immunol. 77 (2) (2008) 152–160.
[9] K.K. Ferguson, et al., Longitudinal Profiling of Inflammatory Cytokines and C-reactive Protein during Uncomplicated and Preterm Pregnancy, Am. J. Reprod. Immunol. 72 (3) (2014) 326–336.
[10] K.K. Ferguson, et al., Repeated measures of inflammation and oxidative stress biomarkers in preeclamptic and normotensive pregnancies, Am. J. Obstet. Gynecol. 216 (5) (2017) 527 e1–527 e9.
[11] C.S. Kronborg, et al., Longitudinal measurement of cytokines in pre-eclamptic and normotensive pregnancies, Acta Obstet. Gynecol. Scand. 90 (7) (2011) 791–796.
[12] A.M. Mitchell, M. Palettas, L.M. Christian, Fetal sex is associated with maternal stimulated cytokine production, but not serum cytokine levels, in human pregnancy, Brain Behav. Immun. 60 (2017) 32–37.
[13] J.J. Reyes-Lagos, et al., Women Serum Concentrations of the IL-10 Family of Cytokines and IFN-gamma Decrease from the Third Trimester of Pregnancy to Active Labor, NeuroImmunoModulation 24 (3) (2017) 162–170.
[14] R. Romero, et al., The maternal plasma proteome changes as a function of gestational age in normal pregnancy: a longitudinal study, Am. J. Obstet. Gynecol. 217 (1) (2017) 67 e1–67 e21.
[15] K.M. Ross, et al., Patterns of peripheral cytokine expression during pregnancy in two cohorts and associations with inflammatory markers in cord blood, Am. J. Reprod. Immunol. 76 (5) (2016) 406–414.
[16] J. van der Giessen, et al., Modulation of cytokine patterns and microbiome during pregnancy in IBD, Gut 69 (3) (2020) 473–486.
[17] S. Vassiliadis, et al., Serum levels of pro- and anti-inflammatory cytokines in non-pregnant women, during pregnancy, labour and abortion, Mediators Inflamm. 7 (2) (1998) 69–72.
[18] M.L. Okun, et al., Disturbed sleep and inflammatory cytokines in depressed and nondepressed pregnant women: an exploratory analysis of pregnancy outcomes, Psychosom. Med. 75 (7) (2013) 670–681.
[19] A.M. Hedman, et al., Longitudinal plasma inflammatory proteome profiling during pregnancy in the Born into Life study, Sci. Rep. 10 (1) (2020) 17819.
[20] V.A. Holmes, et al., Plasma levels of the immunomodulatory cytokine interleukin-10 during normal human pregnancy: a longitudinal study, Cytokine 21 (6) (2003) 265–269.
[21] N. Aghaeepour, et al., An immune clock of human pregnancy, Sci. Immunol. 2 (15) (2017).
[22] E. Brann, et al., Inflammatory and anti-inflammatory markers in plasma: from late pregnancy to early postpartum, Sci. Rep. 9 (1) (2019) 1863.
[23] L.M. Christian, K. Porter, Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: Effects of maternal body mass index, Cytokine (2014).
[24] C. Graham, et al., In vivo immune signatures of healthy human pregnancy: Inherently inflammatory or anti-inflammatory? PLoS ONE 12 (6) (2017), e0177813.
[25] T.A. Kraus, et al., Peripheral blood cytokine profiling during pregnancy and postpartum periods, Am J Reprod Immunol 64 (6) (2010) 411–426.
[26] T.A. Kraus, et al., Characterizing the pregnancy immune phenotype: results of the viral immunity and pregnancy (VIP) study, J. Clin. Immunol. 32 (2) (2012) 300–311.
[27] M. Palm, et al., Involvement of inflammation in normal pregnancy, Acta Obstet. Gynecol. Scand. 92 (5) (2013) 601–605.
[28] Stewart, F.M., et al., Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. 179 (2007. **92**(3): p. 969-75.

[29] T.A. Moore, A.J. Case, Cytokine levels throughout the perinatal period, J. Matern. Fetal. Neonatal. Med. (2021) 1–15.
[30] F. Palm, et al., Progestin treatment does not affect expression of cytokines, steroid receptors, oxytocin receptor, and cyclooxygenase 2 in fetal membranes and endometrium from pony mares at parturition, Theriogenology 79 (1) (2013) 59–68.
[31] La Rocca, C., et al., *The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus.* Immunol Lett, 2014. **162**(1 Pt A): p. 41-8.
[32] A.P. Weetman, Immunity, thyroid function and pregnancy: molecular mechanisms, Nat. Rev. Endocrinol. 6 (6) (2010) 311–318.
[33] K.M. Ross, et al., Immune epigenetic age in pregnancy and 1 year after birth: Associations with weight change, Am. J. Reprod. Immunol. 83 (5) (2020), e13229.
[34] K.M. Ross, et al., Socioeconomic Status, Preeclampsia Risk and Gestational Length in Black and White Women, J. Racial. Ethn. Health Disparities 6 (6) (2019) 1182–1191.
[35] C. Giurgescu, et al., Racial discrimination predicts greater systemic inflammation in pregnant African American women, Appl. Nurs. Res. 32 (2016) 98–103.
[36] S.L. Gillespie, K. Porter, L.M. Christian, Adaptation of the inflammatory immune response across pregnancy and postpartum in Black and White women, J. Reprod. Immunol. 114 (2016) 27–31.
[37] L.S. Keenan-Devlin, et al., Maternal Income during Pregnancy is Associated with Chronic Placental Inflammation at Birth, Am. J. Perinatol. 34 (10) (2017) 1003–1010.
[38] G.E. Miller, et al., Maternal socioeconomic disadvantage is associated with transcriptional indications of greater immune activation and slower tissue maturation in placental biopsies and newborn cord blood, Brain Behav. Immun. 64 (2017) 276–284.
[39] M.S. Finy, L.M. Christian, Pathways linking childhood abuse history and current socioeconomic status to inflammation during pregnancy, Brain Behav. Immun. 74 (2018) 231–240.
[40] J.H. Rowe, et al., Pregnancy imprints regulatory memory that sustains anergy to fetal antigen, Nature 490 (7418) (2012) 102–106.
[41] S.W. Coppack, Pro-inflammatory cytokines and adipose tissue, Proc. Nutr. Soc. 60 (3) (2001) 349–356.
[42] S. Siddiqui, et al., Augmentation of IL-6 production contributes to development of gestational diabetes mellitus: An Indian study, Diabetes Metab. Syndr. 13 (2) (2019) 895–899.
[43] E.A. Enninga, et al., Fetal sex-based differences in maternal hormones, angiogenic factors, and immune mediators during pregnancy and the postpartum period, Am. J. Reprod. Immunol. 73 (3) (2015) 251–262.
[44] R. Romero, et al., The role of infection in preterm labour and delivery, Paediatr. Perinat. Epidemiol. 15 (Suppl 2) (2001) 41–56.
[45] Raudenbush, S.W., A.S. Bryk, and R. Congdon, *HLM 8 for Windows*. 2019, Scientific Software International: Lincolnwood, IL.
[46] J.J. Hox, Multilevel analysis: Techniques and applications, 2 ed., Routledge, Great Britain, 2010.
[47] M. Del Giudice, S.W. Gangestad, Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters, Brain Behav. Immun. 70 (2018) 61–75.
[48] C.A. Hunter, S.A. Jones, IL-6 as a keystone cytokine in health and disease, Nat. Immunol. 16 (5) (2015) 448–457.
[49] F. Atzeni, P. Sarzi-Puttini, Tumor necrosis factor, in: S. Maloy, K. Hughes (Eds.), Brenner's Encyclopedia of Genetics, Elsevier, USA, 2013, pp. 229–231.
[50] K.A. Brennan, J. Zheng, Interleukin 8, in: S.J. Enna, D.B. Bylund (Eds.), xPharm: The Comprehensive Pharmacology Reference, Elsevier, USA, 2008, pp. 1–4.
[51] K.W. Moore, et al., Interleukin-10 and the interleukin-10 receptor, Annu. Rev. Immunol. 19 (2001) 683–765.
[52] S. Pestka, et al., Interleukin-10 and related cytokines and receptors, Annu Rev Immunol 22 (2004) 929–979.
[53] K.N. Couper, D.G. Blount, E.M. Riley, IL-10: The Master Regulator of Immunity to Infection, J. Immunol. 180 (9) (2008) 5771–5777.
[54] S.P. Murphy, S. Sharma, IL-10 and pregnancy, in: G. Mor (Ed.), Immunology of Pregnancy, Springer, USA, 2006, pp. 1–11.
[55] A.J. Lee, A.A. Ashkar, The Dual Nature of Type I and Type II Interferons, Front. Immunol. 9 (2018) 2061.
[56] W. Simpson, et al., Relationship between inflammatory biomarkers and depressive symptoms during late pregnancy and the early postpartum period: a longitudinal study, Braz J Psychiatry 38 (3) (2016) 190–196.
[57] S. Hauguel-de Mouzon, M. Guerre-Millo, The placenta cytokine network and inflammatory signals, Placenta 27 (8) (2006) 794–798.
[58] J.M. Bowen, et al., Cytokines of the placenta and extra-placental membranes: roles and regulation during human pregnancy and parturition, Placenta 23 (4) (2002) 257–273.