Received: 15 June 2016 | Accepted: 15 August 2016

DOI: 10.1111/aji.12563

ORIGINAL ARTICLE

WILEY AJRI American Journal of Reproductive Immunology

Patterns of peripheral cytokine expression during pregnancy in two cohorts and associations with inflammatory markers in cord blood

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Problem: Maternal inflammation undergoes adaptations during pregnancy, and excessive inflammation has been associated with adverse outcomes. One mechanism may be maternal inflammation transmission to the fetal compartment. Links between maternal pregnancy inflammation and fetal inflammation are poorly characterized.

Method: Principal components analysis was used to extract underlying inflammation components across cytokines (IFN-γ, IL-10, IL-13, IL-6, IL-8, TNF-α) in two pregnancy cohorts (SPAH N=87, MOMS N=539) assessed during the second and third trimesters. Links between maternal inflammation over pregnancy and fetal (cord blood) inflammation were assessed.

Results: Substantial cytokine rank-order stability was observed in both cohorts, β's range .47-.96, *P*'s <.001. Two consistent inflammatory components were extracted: a pro-inflammatory (IL-10, IL-6, IL-8, TNF-α, IFN-γ) component and anti-inflammatory

(IL-13) component. Higher maternal pro-inflammatory and lower anti-inflammatory indices during pregnancy were associated with higher cord blood inflammation, *P*'s>.04.

Conclusion: Maternal inflammation indices over pregnancy were associated with inflammation in cord blood at birth. Results have implications for understanding pregnancy inflammatory processes and how maternal inflammation may be transmitted to fetal circulation.

KEYWORDS
cord blood, inflammation, pregnancy

1 | INTRODUCTION

Inflammation plays important roles across all stages of pregnancy,[1] from implantation through labor, delivery, and postpartum recovery. Pregnancy is believed to be a period of immunomodulation, with processes early (i.e., implantation) and late (i.e., labor and delivery) in pregnancy characterized by greater inflammation, or greater pro-inflammatory to anti-inflammatory or Th1 to Th2 activity. In contrast, mid-pregnancy is characterized by lower inflammation, or lower pro-inflammatory to anti-inflammatory or Th1 to Th2 activity.[2] Several studies have modeled inflammation across pregnancy.[3–12] The majority of these included relatively small samples (N<60), focused on a narrow range of markers (IL-6, IL-10, TNF-α), and considered cytokines in isolation, even though cytokines are part of dynamic networks. To account for cytokine inter-relationships, studies in non-pregnant samples have used factor analysis to extract underlying inflammatory activity components.[13,14] These components have been associated with type 2 diabetes risk and cardiovascular events.[15,16] This approach has not yet been adopted in the context of pregnancy. Cytokines also form regulatory feedback loops. Anti-inflammatory cytokines, such as IL-10, inhibit the expression and functions of pro-inflammatory cytokines like IL-6 and TNF-α. The extent of this pro:anti-inflammatory balance can be modeled by calculating the IL-6:IL-10 ratio. Smaller IL-6:IL-10 ratios reflect tighter coupling of pro- and anti-inflammatory cytokines,[17] resulting in overall lower inflammation, and are prospectively associated with better pregnancy outcomes.[18,19]

Inflammation may serve as a mechanism through which maternal infection, dietary patterns, psychosocial stress, and other environmental stimuli contribute to child neurodevelopmental disorder, obesity, and metabolic dysfunction.[20–23] Maternal systemic inflammation could stimulate fetal inflammation directly if cytokines cross the placenta, or indirectly by triggering placenta cytokine release into fetal circulation.[20,21] While a few studies have assessed associations between inflammatory markers in maternal serum and amniotic fluid,[24,25] there is a paucity of data regarding the association of maternal inflammatory markers over pregnancy with fetal inflammation in cord blood.

Our study evaluated inflammatory components during the second and third trimesters, across a range of pro- and anti-inflammatory cytokines. Rank-order stability and within-person change for each inflammatory marker were examined, and principal components analysis (PCA) was used to extract underlying inflammatory components across both studies. Finally, the association between indices of underlying inflammatory activity over pregnancy and cord blood inflammation was assessed.

2 | METHODS

2.1 | Stress, Pregnancy, and Health study

Stress, Pregnancy, and Health study (SPAH) comprised 100 pregnant women recruited from the Center for Maternal and Fetal Health and NorthShore Community Health Clinic, NorthShore University HealthSystem, Evanston Hospital, Evanston, IL (January 2014–July 2015). Eligible participants spoke English, were 18 years or older and 25 weeks or less gestational age (GA) at enrollment, carried a singleton pregnancy, and were expected to deliver at the Evanston Hospital. Because the focus was on inflammation during normal pregnancy, exclusion criteria included known fetal congenital anomaly and/or chromosomal abnormality, and treatment with progesterone after 14 weeks and/or with chronic corticosteroids. Participants provided informed, signed consent, and the protocol was approved by the Institutional Review Boards of Northwestern University and Evanston Hospital, NorthShore University HealthSystem.

Baseline visits were scheduled during the second trimester, between 22'0 and 26'0 weeks GA, and follow-up visits during the third trimester, between 32'0 and 36'0 weeks GA. Of the 100 participants enrolled, three were lost to follow-up, and 10 delivered before 37 weeks. Because of the focus on term pregnancy, and to avoid confounding of any associations with the additional pathology of preterm birth, we excluded the latter subjects, leaving a sample of 87 women. Cord blood was available from 49 of those deliveries. Missing cord blood data were the result of specimen clotting (n=23) or failed collection (n=28).

2.2 | Measurement of Maternal Stress study

Measurement of Maternal Stress study (MOMS) was a multisite project consisting of 723 pregnant women recruited from prenatal clinics

in Pittsburgh, PA, Chicago, IL, Schuylkill County, PA, and San Antonio, TX (June 2013–May 2014). Eligible participants were English speaking, 18 years or older, carrying a singleton pregnancy, and less than 21 weeks GA at enrollment. As in SPAH, women were not eligible if there was known fetal congenital anomaly, chromosomal abnormalities, progesterone treatment after 14 weeks' gestation, and/or chronic corticosteroid treatment. Participants provided informed, signed consent to participate. The Institutional Review Boards of Northwestern University, the Children's Hospital of Philadelphia, the University of Texas Health Sciences Center, and University of Pittsburgh approved the protocol.

Baseline visits took place between 12'0 and 20'6 weeks GA, and follow-up visits, between 32'0 and 35'6 weeks GA. Of the 723 participants enrolled, 130 were lost to follow-up, 31 delivered preterm (before 37 weeks GA), and medical charts were unavailable for 23. Because of the focus on term pregnancy, the final sample available for analysis was 539 women.

2.3 | Inflammatory markers

2.3.1 | Maternal samples

In SPAH, blood was collected via antecubital venipuncture at each maternal assessment into serum-separating Vacutainer tubes (BD Biosciences, Mississuaga, ON, Canada), which were spun within 120 minutes following collection at 1200 RCF for 10 minutes, per manufacturer's instructions. Serum was harvested and stored at −30C until assay. Cytokines were measured by electrochemoluminescent immunoassay on a Meso Scale Discovery (MSD) instrument (SECTOR Imager 2400; Gaithersburg, MD, USA) using the V-PLEX Proinflammatory Panel 1 (10-Plex) plate, which measures concentrations of IL-12p70, IL-13, IL-8, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, and TNF-α. Samples were thawed prior to analysis, centrifuged to remove any solid material, and supernatant was run neat, in duplicate, according to the manufacturer's instructions. Participant samples were batched, and all plates were purchased from the same lot. Signal CVs and data values were inspected. Cytokines with average CVs>10 and/or concentrations below the fit curve for 50% or more of the sample were identified (IL-12p70, IL-1β, IL-2, IL-4) and excluded. These cytokines are present at low concentrations in healthy adult circulation, and other studies of pregnancy have also failed to detect them.[8]

In MOMS, at each assessment, maternal blood was collected via antecubital venipuncture into 10-mL EDTA-coated Vacutainer tubes (BD Biosciences), which were spun within 30 minutes of collection at 2,000 x ***g*** at 4°C for 15 minutes. Plasma was harvested and stored at −80C until assay. Based on SPAH, a custom MSD V-Plex assay was constructed that measured IFN-γ, IL-10, IL-13, IL-6, IL-8, and TNF-α. Participant samples were batched, and all plates were purchased from the same lot. All had average CVs<10 and concentrations above the fit curve for 50% or more of the sample.

Thus, both studies yielded reliable values for IL-6 (intra-assay CV's [CV]<5.54, LLD=0.06 pg/mL), TNF-α (CV<5.00, LLD=0.04 pg/mL), IL-8 (CV<2.97, LLD=0.04 pg/mL), IL-10 (CV<4.63, LLD=0.03 pg/mL), IL-13 (CV<8.87, LLD=0.24 pg/mL), and IFN-γ (CV<5.16, LLD=0.20 pg/mL).

2.3.2 | Cord blood samples

Cord blood samples were collected for SPAH cohort deliveries only. Cord blood was collected following delivery from the umbilical vein into an EDTA-treated Vacutainer tube (BD Biosciences), and centrifuged for 15 minutes at 2,000 x ***g*** at 4C, as per manufacturer's instructions. Plasma was harvested and stored at −70C. Cytokines were assayed in the same manner as above on the V-PLEX Proinflammatory Panel 1 (10-Plex). Concentrations for IL-1β were not detectable for more than 50% of the sample and so were excluded. The nine remaining cytokines included IFN-γ (CV=3.40, LLD=.20 pg/mL), IL-10 (CV=3.48, LLD=0.03 pg/mL), IL-13 (CV=5.69, LLD=0.24 pg/mL), IL-6 (CV=3.76, LLD=0.06 pg/mL), IL-8 (CV=3.40, LLD=0.04 pg/mL), TNF-α (CV=3.91, LLD=0.04 pg/mL), IL-12p70 (CV=8.70, LLD=0.16 pg/mL), IL-2 (CV=8.70, LLD=0.13 pg/mL), and IL-4 (CV=8.75, LLD=0.02 pg/mL). Cytokine distributions were inspected for outliers across assessments and studies. Distributions were positively skewed throughout and were $\log_{10}$ transformed prior to analyses.

2.4 | Analytic strategy

Analyses were conducted using IBM SPSS Statistics 22.[26] Less than 10% of cytokine data was missing across both cohorts and assessments, a rate of missingness which is not likely to introduce bias into analyses.[27] Analyses were also rerun by substituting any missing values by lower limit of detection (LLD)/√2,[4] and pattern of results was unaffected. Analyses were conducted separately for SPAH and MOMS cohorts. To account for "noise" potentially introduced by participant differences in gestational stage at and elapsed time between assessments,[3,4,8] statistical models were adjusted for pregnancy stage at venipuncture (2nd trimester GA at venipuncture, and weeks between 2nd and 3rd trimester assessments). To account for diurnal variation,[28] models also were adjusted for time of day at venipuncture. Pattern of results was unchanged with and without time variables in models.

To assess cytokine stability across pregnancy, linear regression models were used to assess the association between third and second trimester values, adjusting for sample collection timing. The key outcome was the standardized regression coefficient (beta), which relates third trimester to second trimester concentrations adjusting for sample timing. It can be interpreted like a correlation, with values from 0 to 1, and higher values indicating greater rank-order stability. That is, a woman's cytokine values were at similar points in the sample-wide distribution across both assessments, indicating higher stability over time.

To determine whether inflammatory markers changed across pregnancy, linear regression models were used to predict the difference between assessments, controlling for sample collection timing. The key coefficient here was the intercept, which reflects the average change between assessments; positive values denote increases and negative values denote decreases between assessments.

PCA with non-orthogonal rotation was conducted to determine whether inflammatory markers clustered along underlying components. Larger sample sizes increase reliability of these analyses,[29] so

TABLE 1 SPAH and MOMS sample descriptives

	SPAH (N=87)			MOMS (N=539)		
	% (N)	Mean ± SD	Range	% (N)	Mean ± SD	Range
Age (y)		30.5 ± 5.30	18–39		29.6 ± 5.77	18–50
Race						
White	52.2 (47)			62.9 (339)		
Black	25.6 (23)			15.2 (82)		
Hispanic/Latina	16.7 (15)			16.9 (91)		
Other	5.6 (5)			5.0 (27)		
Highest degree						
≤High school	15.5 (14)			24.9 (134)		
Some college	23.3 (21)			33.2 (179)		
≥Bachelor's	61.2 (55)			41.7 (225)		
Gestational age (wk)						
Second trimester		22.9 ± 1.41	19–27		16.5 ± 2.49	12–20
Third trimester		32.6 ± 1.33	30–35		33.7 ± 1.20	32–35
Birth		39.2 ± 1.15	37.0–42.1		39.4 ± 1.09	37–42
Time of blood draw						
Second trimester		12:39 ± 2:42	9:02–18:30		12:23 ± 2.49	7:30–23:00
Third trimester		12:26 ± 2.52	9:00–19:15		12:12 ± 2.62	7:00–22:00

SPAH, Stress, Pregnancy and Health study; MOMS, Measurement of Maternal Stress study.

MOMS components were extracted first, and SPAH was used to confirm the obtained patterns. Identified components were calculated by summing appropriate z-scored inflammatory markers.

Finally, using data from SPAH, we tested whether maternal inflammation and newborn inflammation were related. Correlations were used to determine whether associations existed between maternal calculated inflammatory indices (PCA-derived components and IL-6:IL-10) and cord blood inflammatory markers (results not shown). Significant correlations were followed by linear regression models predicting cord blood cytokine concentrations from maternal inflammatory indices, adjusting for maternal sample collection timing and labor/delivery variables that could affect maternal/fetal inflammation: route of delivery (vaginal/cesarean section) and evidence of infection (present/absent), obtained by examining maternal and newborn medical charts post-delivery. Infection was defined as reporting of fever, chorioamnionitis, endometritis, or another infection (e.g. UTI, MRSA) in maternal or newborn medical charts.

3 | RESULTS

SPAH and MOMS sample characteristics are presented in Table 1. Cytokine concentrations are presented in Table 2.

3.1 | Rank-order stability

There was significant rank-order stability in cytokine expression from the second to third trimester (Table 3). That is, across inflammatory markers assessed, a woman's cytokine values tended to be at similar points in the sample-wide distribution across both assessments, or women who were above-average during the second trimester tended to be above-average during the third trimester as well. SPAH beta coefficients ranged between β_{IL13}=.962 and $\beta_{IL8=}$.628, with average β=.810. MOMS values ranged between β_{IL13}=.942 and $\beta_{IFN\gamma}$=.372, with average β=.590 (Fig. 1).

3.2 | Change across pregnancy

Although rank-order stability was high, many cytokines did change with respect to absolute level of expression (Table S1). Anti-inflammatory markers decreased or showed no significant changes between the second and third trimesters (Fig. 2). IL-13 did not significantly change in both SPAH, *b*(SE)=.014 (.010), *P*=.154, and MOMS, *b*(SE)=.004 (.005), *P*=.430. IL10 decreased in SPAH, *b*(SE)=−.021 (.008), *P*=.012, but did not significantly change in MOMS, *b*(SE)=.006 (.003), *P*=.077.

In contrast, pro-inflammatory markers increased or showed no significant changes between the second and third trimesters. IL-8 increased in both SPAH, *b*(SE)=.044 (.012), *P*=.001, and MOMS, *b*(SE)=.016 (.006), *P*=.007. IL-6 did not significantly change in SPAH, *b*(SE)=.014 (.010), *P*=.154, but increased in MOMS, *b*(SE)=.035 (.004), *P*<.001. TNF-α also did not significantly change in SPAH, *b*(SE)=4.12×10^{-4} (.006), *P*=.944, but increased in MOMS, *b*(SE)=.015 (.003), *P*<.001. The IL-6:IL-10 ratio increased in both SPAH, *b*(SE)=.236 (.076), *P*=.003, and MOMS, *b*(SE)=.216 (.066), *P*=.001. IFN-γ, involved in innate viral immunity, decreased in both SPAH, *b*(SE)=−.095 (.028), *P*=.001, and MOMS, *b*(SE)=−.027 (.011), *P*=.014.

TABLE 2 Maternal (SPAH and MOMS) and cord blood (SPAH) serum cytokine values (log-pg/mL). Differences were calculated by subtracting earlier assessments from later assessments. All calculations and statistics were performed using log_{10}-transformed cytokine values

Cytokine	Assessment	SPAH (Mn ± SD)	MOMS (Mn ± SD)
IFN-γ	2nd trimester	.794 ± .325	.654 ± .213
	3rd trimester	.704 ± .328	.628 ± .191
	Difference	−.090 ± .260	−.025 ± .223
	Cord blood	.765 ± .138	N/A
IL-10	2nd trimester	.149 ± .145	.135 ± .073
	3rd trimester	.131 ± .104	.140 ± .070
	Difference	−.021 ± .081	.005 ± .066
	Cord blood	.219 ± .096	N/A
IL-13	2nd trimester	.534 ± .356	.551 ± .294
	3rd trimester	.530 ± .346	.560 ± .286
	Difference	−.025 ± .089	.007 ± .101
	Cord blood	.713 ± .327	N/A
IL-6	2nd trimester	.204 ± .142	.192 ± .090
	3rd trimester	.218 ± .138	.226 ± .083
	Difference	.015 ± .093	.035 ± .075
	Cord blood	.512 ± .284	N/A
IL-8	2nd trimester	.723 ± .144	.456 ± .132
	3rd trimester	.766 ± .125	.471 ± .118
	Difference	.044 ± .111	.017 ± .120
	Cord blood	.933 ± .353	N/A
TNF-α	2nd trimester	.347 ± .123	.297 ± .066
	3rd trimester	.348 ± .124	.311 ± .064
	Difference	.001 ± .055	.014 ± .061
	Cord blood	.569 ± .075	N/A
IL-6:IL-10	2nd trimester	1.72 ± 1.10	1.96 ± 1.73
	3rd trimester	1.96 ± 1.09	2.20 ± 1.40
	Difference	.249 ± .753	.245 ± 1.36
	Cord blood	2.46 ± 1.26	N/A

SPAH, Stress, Pregnancy and Health study; MOMS, Measurement of Maternal Stress study.
SPAH N's ranged from 100 to 73 and 93 to 70 during the second and third trimesters, respectively. MOMS N's ranged from 539 to 519 and 538 to 522 during the first and second assessments, respectively.

3.3 | Extracting underlying components

To determine whether inflammatory markers formed clusters, PCA analyses were preformed separately at each time point for each study. Results for MOMS second trimester data yielded a two-component solution, which explained 53.7% of the total variance. The first component consisted of TNF-α, IFN-γ, IL-10, IL-6, and IL-8 (pro-inflammatory), and the second component consisted of IL-13 (Table 4). It should be noted that while IL-10 functions as an anti-inflammatory cytokine, it is only expressed under conditions of pro-inflammatory activity. As a consequence, levels of IL-10 correlate positively with more traditional pro-inflammatory markers like IL-6 and TNF,[30] and IL-10 clusters with pro-inflammatory markers in PCA.

Similar patterns emerged for the other PCA analyses, with 54%–72% of the total inflammatory marker variance being accounted for by the two components across time points and cohorts. Communalities for MOM inflammatory markers ranged from .32 to .85 (average=.55), with average factor loadings for the pro-inflammatory component ranging from .50 to .82 (average=.62) and the IL-13 component .92 for both 2nd and 3rd trimester assessments. Communalities for SPAH inflammatory markers ranged from .49 to .84 (average=.70), with average factor loadings for the pro-inflammatory component ranging from .50 to .86 (average=.76) and the IL-13 components from .82 to .90 (average=.86).The pro-inflammatory component was calculated by summing the z-scores of the appropriate inflammatory markers and displayed high rank-order stability, β's>.431 (Table 3).

TABLE 3 Modeling rank-order stability between the second and third trimesters. The beta coefficient can be interpreted as a correlation between earlier and later assessment values, after adjusting for blood draw timing and pregnancy stage at assessment

	2nd to 3rd trimester			
	SPAH		MOMS	
	β	*P*	β	*P*
IFN-γ	.684	<.001	.373	<.001
IL-10	.880	<.001	.508	<.001
IL-13	.962	<.001	.942	<.001
IL-6	.800	<.001	.623	<.001
IL-8	.628	<.001	.486	<.001
TNF-α	.895	<.001	.558	<.001
IL-6:IL-10	.818	<.001	.645	<.001
Pro-inflammatory component	.848	<.001	.431	<.001

SPAH, Stress, Pregnancy and Health study; MOMS, Measurement of Maternal Stress study.

3.4 | Maternal and fetal cytokines

Linear regression models, controlling for timing of maternal sample collection, mode of delivery and evidence of infection at delivery, revealed associations between maternal and fetal cytokine expression (Table S2). Higher average maternal pro-inflammatory component and IL-6:IL-10 were associated with greater cord blood inflammation, while higher IL-6:IL-10 was associated with lower cord blood IL13, *b*(SE)=−.117 (.050), *P*=.024. Higher maternal pro-inflammatory component scores predicted higher cord blood IL-6:IL-10, *b*(SE)=.109 (.050), *P*=.035, IL-6, *b*(SE)=.024 (.008), *P*=.007, and marginally higher IL-8, *b*(SE)=.025 (.014), *P*=.084. In contrast, higher average maternal anti-inflammatory IL-13 predicted lower cord blood inflammation, as indicated by higher cord blood IL-13, *b*(SE)=.188 (.037), *P*<.001, and IFN-γ, *b*(SE)=.048 (.017), *P*=.007, and lower IL-6:IL-10, *b*(SE)=−.404 (.181), *P*=.033.

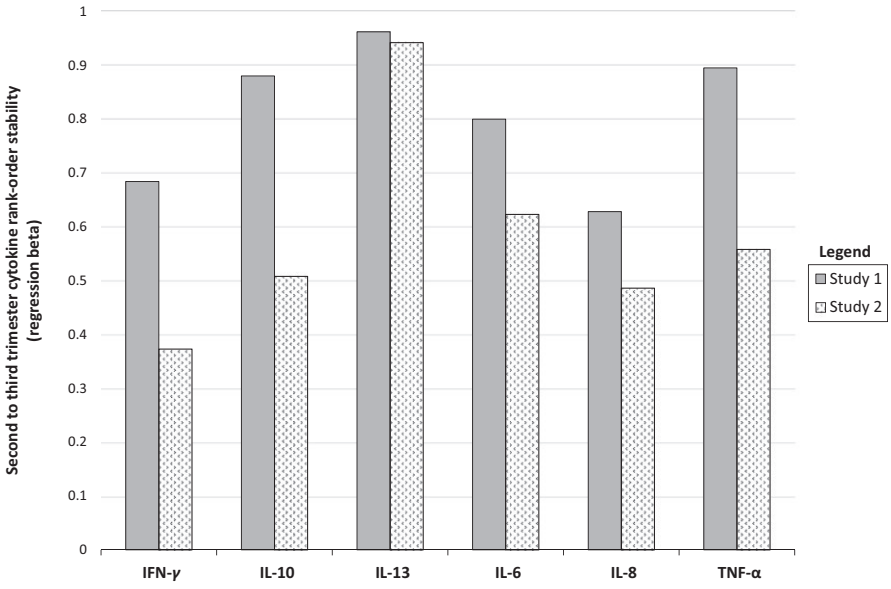

FIGURE 1 Rank-order stability in individual cytokines between the second and third (SPAH: Stress, Pregnancy, and Health study and MOMS: Measurement of Maternal Stress study) trimesters of pregnancy. Plotted values are regression beta coefficients, which can be interpreted like correlations after pregnancy stage and sample timing are considered. Values range from 0 to 1, with higher values indicating greater rank-order stability. All values were significant (P<.05)

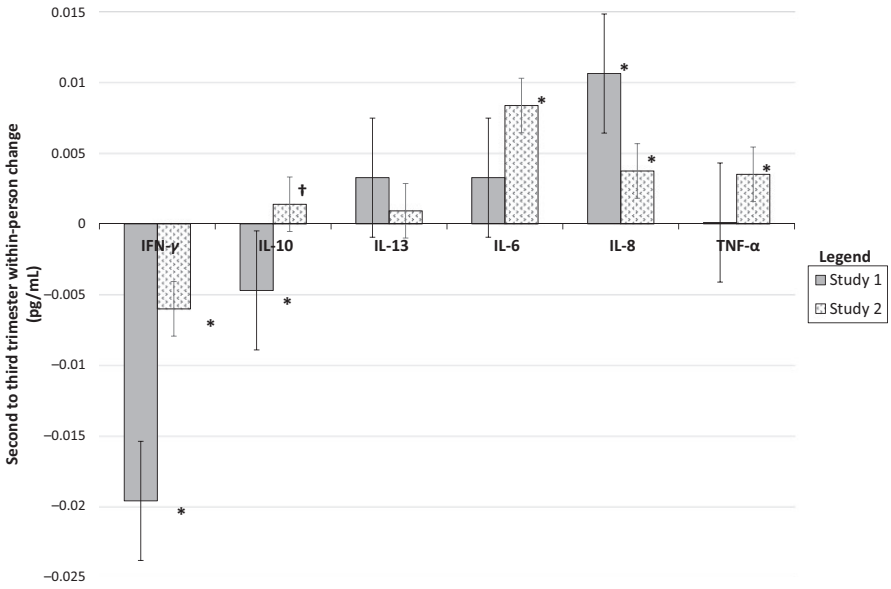

FIGURE 2 Within-person change in individual cytokines between the second and third trimesters in Stress, Pregnancy, and Health (SPAH) study and Measurement of Maternal Stress (MOMS) study, adjusted for time of blood draw and pregnancy stage at both assessments. Bars with an "*"denote statistically significant and with a "†" denote marginally significant values. Positive values indicate increases in within-person cytokine concentrations over the follow-up, and negative, decreases

4 | DISCUSSION

These analyses extend previous work on maternal cytokines during pregnancy by exploring stability, change, and clustering of cytokines from mid-to-late pregnancy. Consistent with past studies,[8] the rank order of cytokine expression was fairly stable across pregnancy; women tended to remain at the same point in the sample distribution over time. These findings suggest that pregnancy inflammatory "levels" are likely determined by pre-pregnancy factors, consistent with past research.[3,31] This highlights the importance of the preconception period in understanding the gestational inflammatory milieu.

Despite rank-order stability, significant within-person fluctuations in most cytokines were observed. Although these fluctuations are generally small, past research suggests they may have clinical relevance.[8] The pro-inflammatory cytokines, IL-6 and TNF-α, generally increased between the second and third trimesters. This converges with past work on IL-6[3–5,9–12] and most studies of TNF-α[3,4,8,9,11,12] (for an exception, see [5]). The chemokine IL-8 increased between the second and third trimesters. Consistent with prior work,[8] IFN-γ, involved in viral resistance, decreased between the second and third trimesters. The anti-inflammatory cytokines IL-13 and IL-10 demonstrated similar patterns, remaining relatively stable or decreasing between the second and third trimesters. Consistent with these patterns, the IL-6:IL-10 ratio increased between the second and third trimesters. Overall, these changes highlight the nuance and complexity in pregnancy inflammatory adaptations, and are consistent with an immunomodulatory view

TABLE 4 Summary of principal components analysis (PCA) over the second and third trimesters in SPAH and MOMS. Components were extracted using a non-orthogonal (oblim) rotation, first in MOMS (larger sample) and confirmed in SPAH. Component loadings for each cytokine can be interpreted like regression coefficients, that is, the unique variance in a given component accounted for by a given cytokine. A two-component solution was yielded across second and third trimester assessments in both SPAH and MOMS. Component 1 generally consisted of TNF-α, IFN-γ, IL-10, IL-6, and IL-8. Component 2 generally consisted of IL-13

	MOMS				SPAH			
	2nd trimester		3rd trimester		2nd trimester		3rd trimester	
Component:	1	2	1	2	1	2	1	2
TNF-α	.818		.817		.805		.830	
IFN-γ	.642		.550		.831		.859	
IL-10	.597		.468	.537	.761		.791	
IL-6	.597		.677		.707		.874	
IL-8	.495		.544		.603	.547	.498	.569
IL-13		.919		.919		.895		.817
Correlations	.132		.185		−.020		−.036	
Variance (%)	35.6	17.9	36.8	19.2	47.3	20.9	53.5	18.1

SPAH, Stress, Pregnancy and Health study; MOMS, Measurement of Maternal Stress study.
Non-orthogonal rotations allow for correlations between extracted components, which are reported above (correlations were non-significant). Variance (%) refers to the amount of total variance accounted for by a given component.

of pregnancy, wherein shifts towards pro-inflammatory states occur mid-to-late pregnancy.[2]

Although generally consistent, there were between-study differences with respect to extent of rank-order stability and within-person change. SPAH rank-order stability estimates were higher, and differences with respect to IL-10, IL-6, and TNF-α change were observed. These are likely due to methodological and sample characteristic differences between studies. Compared to MOMS, SPAH samples were collected during narrower assessment windows, at similar times of the day, and at a single research site. These between-study factors cannot be corrected by controlling for sample collection timing, and so SPAH rank-order stability estimates may reflect less "noise" in data. Differences in cytokine direction of change between studies could be due to differences in statistical power/sample size (N=87 vs 539) or sample characteristics (see Table 1). Future research should explore how individual differences, for example, race or socioeconomic status, predict rank-order stability and within-person change in inflammation over pregnancy.

Using PCA, we found evidence for clustering of the cytokines IFN-γ, IL-10, IL-6, IL-8, and TNF-α. This component likely reflects underlying coordinated pro-inflammatory activity. The component showed rank-order stability across the second and third trimesters, and was associated with cord blood inflammatory markers at delivery, suggesting its potential predictive utility. Modeling rank order stability and within-person change in individual inflammatory markers may yield inconsistent findings across studies due to methodological and sample characteristic differences between studies. PCA-derived inflammatory indices, however, overcome these problems by extracting common, underlying inflammatory components, which are less affected by study-specific idiosyncrasies. As such, these inflammatory components may be more reliable and more predictively useful within the context of pregnancy than considering inflammatory markers separately.

We also found associations between maternal and offspring cytokine concentrations. To the extent that mothers displayed higher pro-inflammatory component values and less tight regulation of IL-6 by IL-10 (higher IL-6:IL-10), neonate cord blood had greater IL-6 and IL-8, less tight regulation of IL-6 by IL-10 (higher IL-6:IL-10), and lower IL-13. Conversely, higher maternal anti-inflammatory IL-13 was associated with tighter regulation of IL-6 by IL-10 (lower IL-6:IL-10), and more IL-13 and IFN-γ in cord blood. Other studies comparing individual inflammatory markers in amniotic fluid and maternal serum reported weak to null associations between inflammatory marker levels in maternal and fetal compartments.[24,25] Here, however, we do observe an association between inflammatory indices in maternal circulation and neonate cord blood. These results again highlight how use of composite indices, calculated across several markers and reflecting underlying inflammatory activity, may be more predictive of outcomes than separately analyzed, individual markers. It remains unclear, however, whether maternal inflammatory cytokines may directly cross the placenta and/or trigger placental inflammation that is transmitted to fetal circulation. Regardless, these analyses suggest that maternal inflammation over pregnancy may be translated to inflammation in fetal circulation. These findings have implications for understanding how maternal infection or elevated inflammatory states may confer risk for fetal neurodevelopmental and other disorders,[20] potentially mediated by corresponding changes in fetal circulating inflammation.

These results must be considered in light of several limitations. First, blood was collected at two time points, during the second and third trimesters only. More frequent assessments would provide a finer-grained look at cytokine dynamics during pregnancy. Second, cord blood samples were only available from a subset (56%) of SPAH deliveries. Third, while the results suggest that maternal and offspring cytokine concentrations are related, neither underlying mechanisms

nor consequences for infant outcomes were explored. Finally, while maternal inflammatory markers were used to calculate indices that could reflect underlying inflammatory regulation, for example, IL-6:IL-10, studies where immune cells are directly stimulated are required to actually test dynamic regulation of the immune system over pregnancy. Future analyses will address maternal determinants of variation in cytokine levels across pregnancy (e.g., demographics, obstetric risk factors, and maternal psychosocial state).

5 | CONCLUSIONS

In sum, this work builds on past research by comparing rank-order stability and within-person change in cytokine expression across two studies of healthy pregnant women, demonstrates that consistent underlying inflammatory components can be seen across assessments and samples, and provides preliminary evidence that maternal cytokine concentrations during pregnancy forecast cord blood milieu at birth. Our findings highlight the complex changes and adaptations that occur in peripheral cytokines over the course of pregnancy and demonstrate that these may be transmitted to the fetus.

ACKNOWLEDGMENTS

This study was made possible with funding from NorthShore University HealthSystem Auxiliary Research Scholar Award, NorthShore Research Career Development Award, Northwestern University Institute for Policy Research and Weinberg College of Arts and Sciences, and Award #HHSN275201200007I—HHSN27500005 of the National Children's Study: Vanguard Study—Task Order 5: Stress and Cortisol Measurement for the National Children's Study (Principal Investigator: Ann E.B. Borders, MD, MSc, MPH). We are grateful for the support of the Evanston Hospital Labor & Delivery nurses and residents for helping with SPAH sample collection, as well the MOMS Study Collaboration including research staff and participants. We also appreciate the important contribution of the MOM-led pilot study collaboration.

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SUPPORTING INFORMATION

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How to cite this article: Ross, K. M., Miller, G., Culhane, J., Grobman, W., Simhan, H. N., Wadhwa, P. D., Williamson, D., McDade, T., Buss, C., Entringer, S., Adam, E., Qadir, S., Keenan-Devlin, L., Leigh, A. K. K. and Borders, A., (2016), Patterns of peripheral cytokine expression during pregnancy in two cohorts and associations with inflammatory markers in cord blood. American Journal of Reproductive Immunology, 76: 406–414. doi: 10.1111/aji.12563