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Original Contribution

Umbilical Cord Serum Cytokine Levels and Risks of Small-for-Gestational-Age and Preterm Birth

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While elevated levels of proinflammatory cytokines are clearly associated with preterm birth, the relation between cytokines and fetal growth is unclear. The authors examined associations between umbilical cord serum cytokine concentrations and risk of small-for-gestational-age (SGA) and preterm birth. This cross-sectional analysis was nested within a National Institute of Child Health and Human Development–University of Alabama population-based cohort study of high-risk prenatal care patients in Jefferson County, Alabama. Patients were enrolled between 1985 and 1988. For 370 singletons, umbilical cord serum concentrations of interferon γ (IFN-γ), tumor necrosis factor α, and interleukins 12p70, 4, and 10 were determined. Associations between each cytokine and SGA and preterm delivery were evaluated using log binomial regression. Increasing log concentration of tumor necrosis factor α was associated with an increased risk of preterm birth (risk ratio (RR) = 2.00, 95% confidence interval (CI): 1.31, 3.06). IFN-γ was associated with a decreased risk of SGA birth (RR = 0.78, 95% CI: 0.61, 1.01). After stratification for preterm birth status, the association between IFN-γ concentration and SGA birth was pronounced among preterm babies (RR = 0.56, 95% CI: 0.31, 1.01). The observations regarding IFN-γ, which is involved in the activation of adaptive immune responses and regulation of trophoblast function, suggest that IFN-γ levels at birth may be related to fetal growth restriction.

cytokines; fetal blood; fetal development; infant, small for gestational age; interferon-gamma; premature birth; tumor necrosis factor-alpha

Abbreviations: AGA, appropriate-for-gestational-age; CI, confidence interval; IFN-γ, interferon γ; PROM, premature rupture of the membranes; RR, risk ratio; SGA, small-for-gestational-age; Th1, T helper 1; TNF-α, tumor necrosis factor α.

Many studies have investigated the role of maternal and fetal inflammation and immunoregulation in the etiology of small-for-gestational-age (SGA) and preterm birth (1, 2). During maternal infection, production of cytokines such as tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ) increases the production of prostaglandins, resulting in early labor (3). It has long been observed that serum protein levels are related to gestational age (4–6). Numerous studies have attempted to determine the relation between cytokines and fetal growth and development (7–22). While positive associations between elevated levels of proinflammatory cytokines (e.g., TNF-α, IFN-γ, and interleukins 1β, 6, and 8) and preterm birth have been reported by a number of investigators (7–13), the evidence on the relation between cytokines and SGA is inconclusive (14–22).

Three small case-control studies (8–15 cases) found that increasing levels of the proinflammatory cytokines TNF-α and interleukin-8 and decreasing levels of the antiinflammatory cytokine transforming growth factor β were associated with an increased risk of intrauterine growth restriction (23–25). Another case-control study (14 cases) found that lower levels of the proinflammatory cytokine interleukin-6 in umbilical cord blood were associated with decreases in birth weight among women with early-onset preeclampsia (26).

In a study by Engel et al. (27, 28), maternal polymorphisms for increased production of proinflammatory

cytokines (TNF-α, interleukin-1, and interleukin-6) were found to be significantly associated with preterm delivery but not with SGA birth. This study further suggested that polymorphisms for ''low-producing'' antiinflammatory cytokines (interleukin-4 and interleukin-10) were associated with a reduced risk of SGA birth and that polymorphisms for ''high-producing'' interleukin-4 were associated with an increased risk of SGA birth (27). In contrast, maternal gene polymorphisms for TNF-α, interleukin-6, and interleukin-10 were not associated with adverse pregnancy outcomes, including SGA birth, in later studies (29–31). However, in none of these studies did investigators measure actual concentrations of cytokines.

Our purpose in this investigation was to examine associations between pro- and antiinflammatory cord blood cytokines and SGA and preterm birth. We hypothesized that increased levels of cytokines in cord blood would be associated with an increased risk of SGA and preterm birth.

MATERIALS AND METHODS

Study design and population

This investigation was nested within the Infant Growth Project, a prospective study of the impact of intrauterine growth restriction on child cognitive function conducted jointly by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the University of Alabama at Birmingham (32, 33). The study was reviewed and approved by the institutional review boards of both institutions. The study design has been described previously (34). In brief, between 1985 and 1988, 3,721 women from an indigent, primarily African-American population of women receiving prenatal care from an Alabama county health department were screened. Pregnant women with a parity of 1 or 2 (i.e., 1 or 2 previous livebirths) who initiated prenatal care at less than 17 weeks' gestation were eligible to participate in the study ($n = 2{,}661$); 1,545 gave written informed consent and were enrolled (33).

Investigators administered questionnaires during each of 3 prenatal clinic visits to collect information on maternal medical history and demographic, socioeconomic, and anthropometric characteristics. The 1,518 singletons delivered during the study period were included in the study cohort, and 893 umbilical cord blood samples were taken. Of these, 370 samples were assayed for cytokines, based on participants' consenting to enroll in a follow-up study on birth outcomes and child cognition (34). Eligible women who enrolled in the study and those who did not enroll were similar with regard to demographic variables (race/ethnicity, age, smoking status, height, and infant birth weight) (34). Additionally, the subcohort of 370 women for this analysis did not significantly differ from the entire Infant Growth Project cohort population in terms of predefined risk factors for SGA birth (previous delivery of an infant weighing less than 2,750 g, previous stillbirth or neonatal death, history of 2 or more spontaneous abortions, history of phlebitis, initial systolic blood pressure above 140 mm Hg, previous preterm birth, prepregnancy weight or weight at the first clinic visit <50 kg, currently smoking or having smoked at conception, and use of alcohol). Mothers reporting smoking at least 1 cigarette per day, on average, at any of the clinic visits were classified as smokers. Alcohol consumption during pregnancy also was measured as a dichotomous variable (yes/no).

Birth outcome assessment

Newborns were weighed within 1 hour of birth. Gestational age was estimated on the basis of the date of the last menstrual period and was confirmed by ultrasonography (usually before 21 weeks). The sonographic estimate of gestational age was used if it differed by at least 2 weeks from the estimate based on the last menstrual period (35). Newborns were classified as SGA if they were below the 10th percentile of weight-for-age based on a fetal growth standard curve (36). Infants were classified as large-for-gestational-age if their weight-for-age was above the 90th percentile. Preterm birth was defined as birth occurring at less than 37 weeks' gestation. Additional information collected at the time of labor and delivery included data on maternal diagnoses that may be associated with inflammation or infection (i.e., diagnosis of preeclampsia ($n = 8$), chorioamnionitis ($n = 4$), or intrapartum fever ($n = 6$)), whether the mother was taking antibiotics ($n = 22$), and whether a preterm delivery was spontaneous or involved premature rupture of the membranes (PROM) ($n = 57$) or was elective ($n = 5$).

Laboratory assessment of cytokines

Umbilical cord blood samples were collected at birth and processed, and an aliquot from each participant ($n = 370$) was shipped on dry ice to the National Institute of Child Health and Human Development, where it was continuously stored at −80°C until all samples were assayed for cytokines in 2001. Concentrations of interleukins 1β, 2, 4–8, 10, and 12p70, TNF-α, IFN-γ, and granulocyte-macrophage colony-stimulating factor were assessed. Cytokines were measured simultaneously in cord serum samples using the Linco immunoassay and the Luminex 100 IS system (Millipore Corporation, Billerica, Massachusetts) (37). Laboratory personnel were blinded to the subjects' pregnancy outcome status. The Luminex system measures concentrations of multiple cytokines in serum samples with bead mapping technology that uses color-coded microscopic beads, each of which is coated with a specific immunoassay reagent. When combined with a sample, the reagents on the surfaces of the beads recognize and capture each specific cytokine through a biochemical binding reaction. Immunoassay results were detected using a Luminex 100 flow-cytometer (38). The standard curve concentration range was between 3.2 pg/mL (lower limit of detection) and 10,000 pg/mL (upper limit of detection).

Five cytokines were present at levels above the lower limit of detection in more than 60% of the samples. These included 3 proinflammatory cytokines (TNF-α, IFN-γ, and interleukin-12p70) and 2 antiinflammatory cytokines (interleukins 4 and 10). The remaining cytokines (interleukins 1β, 2, and 5–8 and granulocyte-macrophage colony-stimulating factor) were present at levels above the lower limit of

detection in fewer than 50% of the samples and were excluded from study analyses (39).

Studies suggest that cytokine levels are stable over time when stored at −80°C (40, 41). To ensure that there was no substantial cytokine degradation in the stored samples, we compared the cytokine concentrations from the Infant Growth Project cohort with those of a similar cohort in Baltimore, Maryland, for which the samples had been stored for only 1 year (see Appendix Table); ranges of values were similar.

Statistical analysis

Cytokines were analyzed as log-transformed continuous variables because their distributions were right-skewed. Cytokine levels below the limit of detection (LOD) were imputed using the formula LOD/$\sqrt{2}$ (42). The percentages of samples with values below the limit of detection were 3% for TNF-α, 20% for IFN-γ, and approximately 33% for interleukins 4, 10, and 12p70. Information on cigarette smoking during pregnancy was missing for 6 participants; these women were coded as nonsmokers. We conducted sensitivity analyses to determine whether imputing missing values for smoking changed effect measures and confidence intervals. We also conducted sensitivity analyses to evaluate whether imputation of cytokine values below the limit of detection using the formula LOD/$\sqrt{2}$ yielded results comparable to those from multiple imputation. We found that both methods yielded similar results, although multiply imputed cytokine values produced somewhat smaller effect measures with wider confidence intervals. We also evaluated cytokine levels in quantiles to ascertain the validity of a log-linear exposure term.

Correlations among log-transformed cytokine levels were evaluated using Spearman rank correlation coefficients. We conducted statistical analyses to assess the associations between cytokines and SGA and preterm birth using separate log-binomial regression models (43) for each individual cytokine, with unadjusted and adjusted models, taking into account the correlation between cytokines. Linear regression models were used to evaluate cytokine levels in relation to gestational age and birth weight as continuous variables. In the models, we adjusted for maternal age, race/ethnicity, prepregnancy weight <50 kg, alcohol consumption, cigarette smoking during pregnancy or at conception, and medical details at the time of labor and delivery as potential confounders. Mode of delivery was not associated with either outcome or any of the cytokine concentrations and was not included as a covariate in our models. We also conducted stratified analyses by SGA status and preterm birth status (preterm birth vs. term birth). Stata 9.2 (Stata Corporation, College Station, Texas) was used for statistical analyses.

RESULTS

There were 72 (19%) preterm deliveries and 75 (20%) SGA births in our study population. Thirteen babies (3.5%) were both preterm and SGA; thus, approximately 18% (13 of 72) of preterm babies were SGA. Demographic characteristics and study variables are listed in Table 1 by SGA status and preterm birth status. Approximately 70% of women were African-American and 30% were Caucasian. African-American women were more likely than Caucasian women to have a preterm birth (22.2% vs. 12.5%; $P = 0.03$). Maternal smoking status and alcohol consumption were associated with an increased risk of SGA birth. The average maternal age was higher among women with SGA infants and lower among women with preterm infants (Table 1).

Umbilical cord serum cytokine concentrations are presented in Table 2. TNF-α, interleukin-12p70, interleukin-10, and interleukin-4 were detected most frequently, and interleukin-6 and interleukin-8 were detected most infrequently. Serum concentrations of the 5 cytokines in this study were significantly positively correlated with each other by Spearman rank correlation coefficients. Interleukin-10 and interleukin-4 had the weakest correlation ($r = 0.22$, $P < 0.001$), and IFN-γ and interleukin-12p70 had the strongest correlation ($r = 0.74$, $P < 0.001$). The correlation between IFN-γ and TNF-α was 0.44 ($P < 0.001$).

Preterm delivery was associated with higher log concentrations of IFN-γ and TNF-α in the unadjusted models. After adjustment for maternal age, race/ethnicity, weight, and smoking status, risk of preterm delivery was associated with increasing log concentrations of IFN-γ (risk ratio (RR) = 1.24, 95% confidence interval (CI): 1.01, 1.52) and TNF-α (RR = 2.18, 95% CI: 1.53, 3.10). Risk ratio estimates decreased after adjustment for maternal medical information (i.e., whether the mother was taking antibiotics or had intrapartum fever) (Table 3). In adjusted linear regression analyses of cytokine levels and gestational age as a continuous variable, a 1-unit increase in log TNF-α concentration was associated with a reduction in gestational age of 6 days (95% CI: −4, −10; $P < 0.01$). After stratification by SGA status, elevated log TNF-α concentrations remained significantly associated with an increased risk of preterm delivery among non-SGA infants (RR = 2.22, 95% CI: 1.56, 3.17), but not among SGA births (RR = 1.12, 95% CI: 0.61, 2.06). Similarly, a log increase in IFN-γ concentration was positively associated with increased risk of preterm delivery among non-SGA births (RR = 1.32, 95% CI: 1.05, 1.66) but not among SGA births (RR = 0.91, 95% CI: 0.49, 1.69). Coding missing values for smoking did not change any of our effect estimates or confidence intervals. Upon evaluating the associations between cytokines and birth outcomes using cytokines as quantiles, we found that the associations between TNF-α and preterm birth and between IFN-γ and SGA birth remained. This was true for both quartiles and quintiles. For tertiles, the 95% confidence intervals widened for the association between IFN-γ and SGA.

In exploratory subanalyses, we found that women who were diagnosed with chorioamnionitis ($n = 4$) or had a fever at the time of labor ($n = 6$) were more likely to have delivered preterm (RR = 4.04 (95% CI: 2.20, 7.43) and RR = 3.62 (95% CI: 1.96, 6.67), respectively). Additionally, women who were receiving antibiotics at the time of delivery ($n = 22$) were also more likely to have delivered preterm (RR = 3.52, 95% CI: 2.30, 5.39).

SGA birth was inversely associated with log concentration of IFN-γ (RR = 0.79, 95% CI: 0.63, 1.00). After

Table 1. Characteristics of Women Selected From the Infant Growth Project for a Study of Cytokines and Small-for-Gestational-Age Birth, by Birth Status, Jefferson County, Alabama, 1985–1988

	SGA Birth ($n = 75$)			Not SGA Birth ($n = 295$)			*P* Value[a]	Preterm Birth ($n = 72$)			Term Birth ($n = 298$)			*P* Value[a]
	Mean (SE)	No.	%	Mean (SE)	No.	%		Mean (SE)	No.	%	Mean (SE)	No.	%	
Maternal age, years	25.3 (4.6)			24.0 (4.2)			0.016	23.3 (4.3)[b]			24.5 (4.3)			0.047
Maternal height, cm	161.8 (6.3)			163.6 (6.5)			0.029	163.5 (5.5)			163.2 (6.7)			0.676
Prepregnancy body mass index[b]	22.9 (5.7)			23.6 (5.9)			0.323	22.3 (4.6)			23.8 (6.1)			0.066
Maternal race/ethnicity							0.144							0.034
Black		59	78.7		207	70.2			59	81.9		207	69.5	
White		16	21.3		88	29.8			13	18.1		91	30.5	
Parity							0.455							0.144
1		52	69.3		191	64.7			42	58.3		201	67.5	
2		23	30.7		104	35.3			30	41.7		97	32.5	
Maternal education							0.380							0.459
Less than high school diploma		25	33.3		98	33.2			21	29.2		102	34.2	
High school diploma		30	40.0		96	32.5			29	40.3		97	32.6	
≥1 year of college		20	26.7		100	33.9			22	30.5		98	32.9	
Missing data					1							1		
Prepregnancy weight <50 kg		21	28.0		69	23.4	0.406		19	36.4		71	23.8	0.649
Cigarette smoking during pregnancy		42	56.0		117	39.7	0.011		24	33.3		135	45.3	0.066
Alcohol consumption during pregnancy		32	42.7		86	29.2	0.025		20	27.8		98	32.9	0.404
Previous infant birth weight <2,750 g		38	50.7		117	39.7	0.085		51	70.8		104	34.9	<0.001
Previous preterm birth		15	20.0		51	17.3	0.584		30	41.7		36	12.1	<0.001
Female infant		40	53.3		139	47.1	0.336		37	51.4		142	47.7	0.569

Abbreviations: SE, standard error; SGA, small-for-gestational-age.
[a] *P* values were calculated by *t* statistic or chi-squared statistic and are 2-sided.
[b] Weight (kg)/height $(m)^2$.

adjustment for race/ethnicity, weight, smoking, alcohol consumption during pregnancy, preeclampsia, and chorioamnionitis, the estimate remained virtually unchanged (Table 4). Excluding infants born large for gestational age ($n = 19$) did not change the results (data not shown). In linear regression analyses of cytokine levels and birth weight as a continuous variable, a 1-unit increase in log IFN-γ concentration was associated with an increase in

Table 2. Cytokine Concentrations in Umbilical Cord Serum ($n = 370$), Infant Growth Project, Jefferson County, Alabama, 1985–1988

Cytokine	Geometric Mean, pg/mL	Percentile					% of Samples With Values Less Than Limit of Detection
		10th	25th	50th	75th	90th	
Interleukin-4	9.43	3.40	5.64	9.65	14.88	26.96	37
Interleukin-6	10.03	1.22	2.57	6.92	29.24	191.37	48
Interleukin-8	7.03	0.71	1.92	5.53	21.07	100.78	55
Interleukin-10	3.98	1.37	2.53	4.19	6.28	11.8	38
Interleukin-12p70	3.56	1.21	2.76	4.12	5.57	7.32	34
Interferon γ	5.65	0.74	3.35	7.04	15.01	24.93	20
Tumor necrosis factor α	8.76	5.15	6.92	8.82	11.55	14.53	3
Granulocyte-macrophage colony-stimulating factor	1.03	0.27	0.47	1.34	1.99	3.18	74

Table 3. Risk Ratios for Preterm Birth in Relation to Umbilical Cord Serum Log Cytokine Levels, Infant Growth Project, Jefferson County, Alabama, 1985–1988

	Model 1[a] ($n = 370$)		Model 2[b] ($n = 370$)		Model 3[c] ($n = 319$)	
	RR	95% CI	RR	95% CI	RR	95% CI
Interferon γ	1.26*	1.02, 1.55	1.24*	1.01, 1.52	1.16	0.93, 1.45
Tumor necrosis factor α	2.00*	1.53, 2.62	2.18*	1.53, 3.10	2.00*	1.31, 3.06
Interleukin-12p70	1.32	0.90, 1.92	1.25	0.86, 1.82	1.16	0.77, 1.76
Interleukin-4	1.09	0.89, 1.33	1.06	0.87, 1.29	1.10	0.89, 1.36
Interleukin-10	1.27*	1.02, 1.57	1.26*	1.01, 1.57	1.18	0.96, 1.46

Abbreviations: CI, confidence interval; RR, risk ratio.
* $P < 0.05$.
[a] Results were unadjusted.
[b] Results were adjusted for maternal age, race/ethnicity, weight, and smoking.
[c] Results were adjusted for smoking, use of antibiotics, and intrapartum fever.

birth weight of 51.8 g (95% CI: 2.3, 101.3; $P = 0.04$), after adjustment for gestational age. After stratification for preterm delivery status, the association between elevated IFN-γ log concentrations and decreased risk of SGA birth was pronounced among preterm births (for preterm births, RR = 0.56, 95% CI: 0.31, 1.01; for term births, RR = 0.86, 95% CI: 0.67, 1.11) (Table 5).

Stratifying by type of preterm delivery, data were available for 62 of the 72 preterm births. SGA preterm babies had lower geometric mean levels of proinflammatory cytokines than did appropriate-for-gestational-age (AGA) preterm babies among spontaneous preterm births and preterm births due to PROM, but not among indicated preterm births (Table 6).

We also examined the association between cytokine levels in cord blood and ponderal index among term births and among all births. We did not find a significant association between any of the cytokines and ponderal index. Serum levels of IFN-γ and interleukin-12p70 were positively associated with birth weight, birth length, and head circumference among babies born at term, but the associations were not statistically significant (data not shown).

DISCUSSION

Higher levels of proinflammatory TNF-α were associated with preterm delivery, which is consistent with prior studies (8, 12, 28). TNF-α may be a biomarker for inflammation or infection, a risk factor for preterm delivery (12). TNF-α may trigger preterm delivery by inducing production of prostaglandin, which in turn induces labor (8). In our study population, signs of infection or inflammation (i.e., chorioamnionitis, intrapartum fever, or use of antibiotics at the time of delivery) were more likely to occur among preterm births.

Unexpectedly, higher levels of T helper 1 (Th1) proinflammatory cytokines (IFN-γ and interleukin-12p70) were associated with a decreased risk of SGA birth, particularly among preterm births. Analyses that stratified results by preterm birth status suggested that the association between IFN-γ concentration and decreased risk of SGA birth was strongest for preterm births. Additionally, interleukin-12p70 concentrations were associated with a decreased risk of SGA birth among preterm births. Thus, among preterm births, we have evidence for a protective role of IFN-γ and interleukin-12p70 with regard to fetal growth

Table 4. Risk Ratios for Small-for-Gestational-Age Birth in Relation to Umbilical Cord Serum Log Cytokine Levels, Infant Growth Project, Jefferson County, Alabama, 1985–1988

	Model 1[a] ($n = 370$)		Model 2[b] ($n = 370$)		Model 3[c] ($n = 338$)	
	RR	95% CI	RR	95% CI	RR	95% CI
Interferon γ	0.79*	0.63, 1.00	0.83	0.66, 1.03	0.78*	0.61, 1.01
Tumor necrosis factor α	1.01	0.65, 1.55	0.99	0.68, 1.43	1.00	0.68, 1.47
Interleukin-12p70	0.81	0.54, 1.23	0.85	0.58, 1.25	0.77	0.50, 1.18
Interleukin-4	0.86	0.69, 1.07	0.89	0.72, 1.09	0.87	0.69, 1.09
Interleukin-10	0.88	0.65, 1.19	0.86	0.65, 1.14	0.90	0.67, 1.22

Abbreviations: CI, confidence interval; RR, risk ratio.
* $P < 0.10$.
[a] Results were unadjusted.
[b] Results were adjusted for race/ethnicity, weight, smoking, and alcohol consumption.
[c] Results were adjusted for the variables listed for model 2, as well as preeclampsia and chorioamnionitis.

Table 5. Risk Ratios[a] for Small-for-Gestational-Age Birth in Relation to Umbilical Cord Serum Log Cytokine Levels, by Preterm Birth Status, Infant Growth Project, Jefferson County, Alabama, 1985–1988

	Term Births[b] (n = 298)		Preterm Births[c] (n = 72)	
	RR	95% CI	RR	95% CI
Interferon γ	0.86	0.67, 1.11	0.56	0.31, 1.01
Tumor necrosis factor α	1.16	0.72, 1.86	0.45	0.13, 1.56
Interleukin-12p70	0.93	0.60, 1.47	0.44	0.15, 1.23
Interleukin-4	0.86	0.68, 1.09	0.88	0.49, 1.57
Interleukin-10	0.93	0.67, 1.29	0.72	0.34, 1.54

Abbreviations: CI, confidence interval; RR, risk ratio.

[a] Results were adjusted for race/ethnicity, smoking, and alcohol consumption.

[b] Among the 298 term births, 62 were small-for-gestational-age births.

[c] Among the 72 preterm births, 13 were small-for-gestational-age births.

restriction. Norwegian investigators found that increasing levels of proinflammatory interleukin-6 in cord blood were associated with a lower risk of restricted fetal growth related to preeclampsia (26), but no prior studies on relations with IFN-γ and interleukin-12p70 have been published.

There are several possible explanations for these associations. Higher concentrations of Th1 proinflammatory cytokines in the fetus may be an indication of a more robust immune response that can be mounted by a larger and better-nourished fetus (44); in other words, reverse causality is possible. However, lower levels of Th1 cytokine concentrations in cord blood could also be a biomarker of placental insufficiency, which in turn is associated with SGA growth. IFN-γ is a Th1 cytokine produced in villous trophoblasts in the placenta, and it plays a role in the activation of adaptive immune responses and regulation of trophoblast function (45). Lower levels of IFN-γ may be an indication of placental insufficiency and impairment of trophoblast function.

Preterm AGA babies had higher levels of Th1 cytokines than did preterm SGA babies. The etiology of preterm birth may differ for SGA and AGA preterm babies. Since a major cause of preterm delivery is infection or inflammation (3), babies born preterm because of infection or inflammation may be born before they have the opportunity to become growth-restricted later in gestation. Some preterm deliveries are elective, initiated for the welfare of the mother or the infant. Babies delivered preterm on an elective basis may already be growth-restricted. We examined whether levels of cytokines varied between SGA and AGA babies in 2 different preterm delivery categories: spontaneous or PROM preterm delivery and indicated preterm delivery. Spontaneous and PROM preterm deliveries are more likely to be associated with infection or inflammation (46). We found that cytokine levels were higher in AGA babies than in SGA babies among spontaneous and PROM preterm deliveries but not among indicated preterm deliveries. Furthermore, there was a higher percentage of SGA babies among indicated preterm births (40%) as compared with spontaneous or PROM preterm births (15%–20%).

Our cross-sectional study design entailed several limitations. Because we measured cytokines at the time of birth, we do not know whether they were biomarkers of the outcomes or biomarkers of underlying processes that were associated with the outcomes. Both are plausible. The advantage of measuring cytokine levels in cord blood is that we were able to measure concentrations in the fetus

Table 6. Geometric Mean Levels of Proinflammatory Cytokines in Small- and Appropriate-for-Gestational-Age Preterm Births ($n = 62$)[a], by Reason for Preterm Delivery, Infant Growth Project, Jefferson County, Alabama, 1985–1988

	Small-For-Gestational-Age Birth			Appropriate-For-Gestational-Age Birth			P Value[b]
	No.	GM, pg/mL	95% CI	No.	GM, pg/mL	95% CI	
Interleukin-12							
Spontaneous/PROM preterm birth	8	3.3	2.4, 4.8	49	4.5	3.9, 5.3	0.06
Indicated preterm birth	2	3.7	1.2, 11.6	3	5.9	2.2, 11.1	0.34
Interferon γ							
Spontaneous/PROM preterm birth	8	3.3	2.1, 5.2	49	8.4	6.4, 11.0	<0.01
Indicated preterm birth	2	6.2	0.6, 60.3	3	9.9	2.2, 44.3	0.36
Tumor necrosis factor α							
Spontaneous/PROM preterm birth	8	7.9	6.9, 9.2	49	10.9	9.7, 12.3	0.02
Indicated preterm birth	2	12.6	11.4, 13.9	3	12.3	11.2, 13.4	0.64

Abbreviations: CI, confidence interval; GM, geometric mean; PROM, premature rupture of the membranes.

[a] There were 72 preterm births in the study population. However, for 10 of these births, information on the type of preterm delivery was missing. There was 1 large-for-gestational-age preterm baby, who was grouped with appropriate-for-gestational-age births.

[b] P value was calculated using Fisher's exact test.

at birth. In previous studies that evaluated maternal polymorphisms or concentrations in amniotic fluid during the second trimester, investigators could not examine the associations with actual concentrations in the fetus at birth. Ideally, we would have been able to assess inflammatory status throughout pregnancy using a variety of measures, including maternal medical records as well as cytokines and other biomarkers of inflammation in maternal serum, amniotic fluid, and cord blood. We also would have liked to have serum samples from the mother in order to compare serum cytokine levels between the mother and the fetus. This would have allowed us to determine whether the immune manifestations were those of the mother or the fetus.

Our findings suggest that elevated levels of the proinflammatory cytokine TNF-α are associated with an increased risk of preterm delivery. However, this association may reflect reverse causality (production of TNF-α as a consequence of being delivered early) or (less likely) the possibility that babies have higher levels of serum TNF-α earlier in gestation. Additionally, our data suggest that higher levels of the Th1 cytokines IFN-γ and interleukin-12p70 in cord blood are associated with a decreased risk of SGA birth among preterm infants. However, this association may reflect reverse causality (larger babies or mothers with better placentas have a more robust immune response) or may be a consequence of differing etiologies for preterm delivery. Our study was too small to explore these associations by type of preterm delivery, because there were only 5 cases of indicated preterm delivery. In future studies, researchers investigating the role of cytokines in fetal growth should include a larger number of indicated preterm deliveries in order to have an appropriate comparison group. Moreover, in future studies, researchers could measure a broader array of serum proteins to investigate whether larger babies have higher levels of serum cytokines and other proteins and substrates, perhaps an indication that they are better nourished. Additionally, future investigators should collect more detailed information on the mother's medical status at the time of labor and delivery, which could affect cytokine levels at the time of birth.

The observations in this study regarding IFN-γ, which has roles in activation of adaptive immune responses and regulation of trophoblast function, suggest that IFN-γ levels at birth may be related to fetal growth restriction.

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Appendix Table. Cytokine Concentrations[a] in Umbilical Cord Serum ($n = 272$), THREE Study, Baltimore, Maryland, 2004–2005

Cytokine	Geometric Mean, pg/mL	Percentile				
		10th	25th	50th	75th	90th
Interleukin-1β[b]	0.45	0.15	0.29	0.47	0.68	0.99
Interleukin-2	1.76	0.94	1.33	1.81	2.39	3.12
Interleukin-6	4.94	1.88	2.59	3.67	6.50	17.38
Interleukin-8	8.08	3.77	5.00	6.97	10.64	17.18
Interleukin-10[b]	1.41	0.62	0.94	1.48	2.20	2.78
Interleukin-12p70	1.87	1.15	1.43	1.88	2.41	2.97
Interferon-γ	1.95	1.17	1.44	1.85	2.58	3.47
Tumor necrosis factor-α	4.61	3.35	3.95	4.52	5.40	6.28
Granulocyte-macrophage colony-stimulating factor	0.80	0.40	0.55	0.75	1.12	1.59

Abbreviation: THREE, Tracking Health Related to Environmental Exposures.

[a] Supplied for comparison with the data shown in Table 2.

[b] For interleukin-1β and interleukin-10, data on 2 subjects were missing.