



## Original Contribution

# Child Intellectual Development in Relation to Cytokine Levels in Umbilical Cord Blood

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Although cytokines play a dual role in the developing neurologic system and in prenatal immune reactions, relations between fetal cytokine levels and child intellectual development remain unknown. The authors investigated associations between umbilical cord serum cytokine concentrations and intellectual outcomes in 369 children within a prospective cohort study, the Eunice Kennedy Shriver National Institute of Child Health and Human Development–University of Alabama Infant Growth Study (1985–1988). Concentrations of interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukins 4, 10, and 12p70 were determined. The Wechsler Preschool and Primary Scale of Intelligence–Revised was administered at age 5 years, producing verbal and performance intelligence quotients (VIQ and PIQ); associations with each cytokine were evaluated using linear and logistic regression. Log-unit increases in IFN- $\gamma$  (adjusted odds ratio (aOR) = 0.67, 95% confidence interval (CI): 0.46, 0.98) and interleukin-12p70 (aOR = 0.43, 95% CI: 0.21, 0.87) were inversely associated with low PIQ (score <70). One log-unit increase in TNF- $\alpha$  was associated with a reduced odds ratio for low VIQ (score <70) among preterm children (aOR = 0.11, 95% CI: 0.01, 0.94) and an elevated odds ratio for low VIQ among small-for-gestational-age children (aOR = 3.96, 95% CI: 0.99, 15.9). IFN- $\gamma$ , which is involved in neurogenesis and perinatal adaptive immunity, may be related to fetal neurologic development overall, while TNF- $\alpha$  may be a marker of intellectual development in vulnerable subgroups.

child development; cohort studies; cytokines; intelligence tests; interferon-gamma; tumor necrosis factor-alpha; umbilical cord

Abbreviations: CI, confidence interval; IFN- $\gamma$ , interferon- $\gamma$ ; IL, interleukin; IQ, intelligence quotient; OR, odds ratio; PIQ, performance intelligence quotient; SGA, small for gestational age; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; VIQ, verbal intelligence quotient.

Severe infant neurologic illness, such as white matter lesions and cerebral palsy (1), and later-life psychiatric illnesses like schizophrenia (2, 3) have been shown to be related to prenatal infection, especially among early preterm births (4–7). Few studies have involved measurement of cytokines, and the authors of these studies have suggested that proinflammatory cytokines, like interleukin (IL)-6 (8), may be mediators for the observed associations (7, 9–11). Because cytokines play a dual role in brain function (12) and in-utero immune responses (9, 13), they are thought to mediate the complex interplay between normal and pathologic prenatal immune processes and critical processes of fetal neurodevelopment

(14–20), thereby possibly influencing long-term nervous system function.

Experimental studies have shown that a number of cytokines are involved in neurogenesis, synaptic maturation, and the generation of neuronal networks and other important processes of brain development (12) and that peripheral cytokines may directly or indirectly affect cognition (9, 12). Both overexpression and deficiency of certain cytokines can positively and negatively influence hippocampus-related memory and synaptic plasticity under pathologic and physiologic conditions, particularly in aging processes (21).

Certain cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are common immune and neuronal cell signaling proteins (16) and have been shown to pass through the blood-brain barrier via active transport mechanisms or indirectly (22, 23); thus, they may mediate bidirectional brain-immune communication across the blood-brain barrier during fetal development (18, 24). The TNF- $\alpha$  signal has been shown to exert protective effects in mice experiments (25) and may play a role in learning and memory by promoting synaptic plasticity (26), suggesting, in turn, that disruption of these processes may lead to cognitive impairment. In animal models, interferon- $\gamma$  (IFN- $\gamma$ ) has been shown to enhance neurogenesis and to improve spatial learning and memory—processes critical for intellectual development in early life (27). Thus, scenarios involving cytokines during the prenatal period may influence later intellectual abilities (9, 12–14, 20).

While insights into the interface of in utero cytokine function and central nervous system processes have steadily increased, mostly on the basis of animal models (15), human longitudinal data for elucidating whether in utero cytokine concentrations at birth are related to intellectual development in children are scarce to date. A body of research has evolved around the role of prenatal cytokines as markers of risk for preterm birth (28) and small-for-gestational-age (SGA) birth (29, 30). Neta et al. (29) previously reported that an increase in TNF- $\alpha$  concentration was associated with a 2-fold increase in the risk of preterm birth and that IFN- $\gamma$  was inversely associated with the risk of SGA birth, especially among preterm births.

We thus hypothesized that umbilical cord blood concentrations of circulating inflammatory cytokines may be related to child intellectual development and that preterm birth and SGA status, respectively, may moderate the relation between levels of certain cytokines at birth and intellectual function in children. We investigated associations between cytokines in umbilical cord serum and measures of intellectual function assessed prospectively among children followed from early pregnancy to the age of 5 years.

## MATERIALS AND METHODS

### Study design and population

This investigation was nested within the Infant Growth Project, a longitudinal study conducted by the Eunice Kennedy Shriver National Institute of Child Health and Human Development and the University of Alabama at Birmingham that was originally designed to determine associations between being born SGA at term and child cognitive function (31–33). The study population has been described previously (31–33). Briefly, investigators screened 3,721 women from a low-income, primarily African-American population who were receiving prenatal care from an Alabama county health department between 1985 and 1988. Of 2,661 eligible women with parity 1 and 2 who sought care at less than 17 weeks of gestation, 1,545 gave written informed consent and were enrolled in the original SGA study (31). Of this original cohort, 27 women had twin pregnancies that were excluded and 27 were lost to follow-up; among the remaining singleton births, 893 umbilical cord blood samples were taken. Of the

pregnancy cohort, 983 pregnancies were eligible for follow-up (33) and 692 (70.4%) children were tested at the age of 5 years (32); 369 also had cord blood samples available that were assayed for cytokines. The 369 subjects who were included in the current study and those who had been included in the original pregnancy cohort were similar with regard to demographic variables, birth weight, and other predefined risk factors for SGA (see Web Table 1, which is posted on the *Journal's* Web site (<http://aje.oxfordjournals.org/>)) (29). The study was reviewed and approved by the institutional review boards of the National Institute of Child Health and Human Development and the University of Alabama at Birmingham.

### Pregnancy and birth outcomes

During each of 3 prenatal clinic visits, information on maternal medical history and demographic, socioeconomic, and anthropometric characteristics was collected by questionnaire. Gestational age was estimated on the basis of the last menstrual period and was confirmed by ultrasound scanning (usually before 21 weeks). The ultrasound information was used if the estimates differed by 2 weeks or more from the last menstrual period. Preterm birth was defined as birth at less than 37 weeks of gestation. Infants were classified as SGA if they were below the 10th percentile of weight-for-age based on a fetal growth standard (34). Additional information collected at the time of delivery included maternal diagnoses that might be associated with inflammation or infection (i.e., diagnosis of preeclampsia, chorioamnionitis, intrapartum fever, or hypertension) and whether the mother was taking antibiotics during labor. Women who reported smoking at least 1 cigarette per day on average at any of the prenatal clinic visits were classified as smokers. Alcohol consumption during pregnancy was measured as a dichotomous variable (yes/no). Information on maternal height and prepregnancy weight, education, and household income was ascertained with a self-administered questionnaire at the first prenatal visit (31).

### Assessment of intellectual function

Intellectual function was assessed at the age of 5 years with the Wechsler Preschool and Primary Scale of Intelligence–Revised, producing verbal and performance intelligence quotients (VIQ and PIQ, respectively) calculated on the basis of sets of subtests assessing verbal and nonverbal abilities (35). Testing was performed at the University of Alabama at Birmingham by 2 evaluators who were extensively trained in the assessment methods and had no knowledge of SGA birth status or any other factors assessed in the study; testing procedures and the results were reviewed regularly by 2 clinical psychologists. In this investigation, limitations in intellectual function were defined as scores less than 70 in VIQ or PIQ, based on the American Association on Intellectual and Developmental Disabilities' current definition of “intellectual disability” (36). Maternal receptive language was measured using the Peabody Picture Vocabulary Test–Revised (37), and the quality of the child's home environment was assessed with the Home Screening Questionnaire (38).

## Measurement of cytokine concentrations

Cord blood samples were collected at birth. From each participant, an aliquot was transported on dry ice to the laboratory of the National Institute of Child Health and Human Development (Bethesda, Maryland), where samples were continuously stored at  $-80^{\circ}\text{C}$  until the time of cytokine analysis. All samples were analyzed for cytokine levels simultaneously in 2001. Concentrations of IL-1 $\beta$ , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, TNF- $\alpha$ , IFN- $\gamma$ , and granulocyte-macrophage colony-stimulating factor were measured simultaneously using the Linco Immunoassay and Luminex 100 IS (Millipore Corporation (Billerica, Massachusetts) and Luminex (Austin, Texas)) based on bead mapping technology using color-coded microscopic beads coated with a specific immunoassay reagent, as reported elsewhere (29). The reagents on the surfaces of the beads recognize and capture specific cytokines through a biochemical binding reaction. Immunoassay results were detected using a Luminex 100 flow cytometer (Millipore Corporation and Luminex). Laboratory technicians were blinded to the outcome status of subjects. The reference curve concentration range lies between 3.2 pg/mL (the lower limit of detection) and 10,000 pg/mL (the upper limit of detection). Cytokines with more than 60% of the samples with levels greater than the lower limit of detection included 3 proinflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$ , and IL-12p70) and 2 antiinflammatory cytokines (IL-4 and IL-10), which were further evaluated. The remaining cytokines had fewer than 50% of samples above the lower limit of detection and were not considered (39). Studies suggest that cytokine levels are stable over time when stored at  $-80^{\circ}\text{C}$  (40, 41). To ensure that there was no substantial cytokine degradation in the stored samples, we compared the cytokine concentrations from the Alabama Infant Growth Project cohort with those of a similar cohort in Baltimore, Maryland, for which the samples had been stored for only 1 year; the ranges of values were similar (29).

## Statistical analysis

Because cytokine concentrations were right-skewed, they were analyzed on a logarithmic scale. Cytokine concentrations below the limit of detection were imputed using the limit of detection divided by the square root of 2 (42). Details of the distribution and concentrations of cytokines have been reported previously for the sample of 370 subjects (29). For this study ( $n = 369$ ), the percentages of samples below the limit of detection were 3% for TNF- $\alpha$ , 20% for IFN- $\gamma$ , 34% for IL-12p70, 39% for IL-10, and 37% for IL-4, with geometric mean concentrations (pg/mL) of 8.76 for TNF- $\alpha$ , 5.92 for IFN- $\gamma$ , 3.97 for IL-12p70, 4.40 for IL-10, and 8.24 for IL-4.

All potentially relevant variables were selected a priori and evaluated for associations with cytokines and the intellectual function measures, respectively, including: maternal age, race/ethnicity (African-American/black vs. non-Hispanic white), maternal education (less than high school, high school, or high school plus 1 year of college), maternal Peabody Picture Vocabulary Test-Revised score, poverty (income  $< \$9,600/\text{year}$ ) (43), Home Screening Questionnaire score, smoking/alcohol

drinking during pregnancy, child gender, type of labor (e.g., induced), cesarean delivery (yes/no), birth weight, preterm birth ( $< 37$  weeks), SGA birth (34), use of antibiotics during labor, intrapartum fever ( $> 100.4^{\circ}\text{F}$ ), chorioamnionitis, hypertension, and preeclampsia. In the final adjusted models, we retained those variables as covariates that were associated with at least one of the cytokines and the intelligence quotient (IQ) measures. Geometric mean values for the cytokine concentrations were calculated according to VIQ ( $< 70$  vs.  $\geq 70$ ) and PIQ ( $< 70$  vs.  $\geq 70$ ) (36), and the differences were assessed using *t* tests. To assess the associations between each log-cytokine value and VIQ and PIQ, respectively, separate unadjusted and adjusted linear and logistic (for categorical outcomes) regression models were fitted. To investigate whether preterm birth ( $< 37$  weeks vs.  $\geq 37$  weeks) or SGA status modified relations between the cytokines and intellectual function, we conducted analyses stratified by preterm birth status (preterm vs. term) and SGA birth status (SGA vs. non-SGA). Stata 11.0 was used for all statistical analyses (StataCorp LP, College Station, Texas).

## RESULTS

Basic characteristics of the study population are shown in Table 1. Average birth weight and gestational age were in the normal range; the percentage of African Americans was 72%. None of the variables indicating maternal inflammation—that is, preeclampsia ( $n = 8$ ), chorioamnionitis ( $n = 4$ ), intrapartum fever ( $n = 6$ ), hypertension ( $n = 22$ ), use of antibiotics during labor ( $n = 22$ ), self-reported maternal infections ( $n = 61$ ), or cesarean delivery ( $n = 52$ )—were related to VIQ or PIQ.

The geometric mean concentrations of the proinflammatory cytokines IFN- $\gamma$  and IL-12p70 were significantly lower among children with low ( $< 70$ ) PIQ scores than among those with higher PIQ scores (for IFN- $\gamma$ , 4.7 pg/mL vs. 6.1 pg/mL ( $P < 0.05$ ); for IL-12p70, 3.4 pg/mL vs. 4.0 pg/mL ( $P < 0.05$ )) (Table 2).

Associations between cytokine concentrations and limitations in intellectual function, that is, scoring less than 70 (reference category:  $\geq 70$ ) (36), were investigated in unadjusted and adjusted logistic regression models; the latter included birth weight, gender, race/ethnicity, and smoking (VIQ only). An increase of 1 log-unit in the proinflammatory cytokines IFN- $\gamma$  and IL-12p70 was associated with approximately 30%–50% reduced odds of scoring less than 70 for PIQ (Table 3). For IL-4, the effect estimate was similar in magnitude to that for IFN- $\gamma$ , but the 95% confidence interval was wider ( $P = 0.07$ ). There was no association between the 5 cytokines and a VIQ test score less than 70 among all subjects. Using the intellectual outcomes measured as continuous variables in adjusted linear regression models, no significant ( $P < 0.05$ ) associations with the log cytokines were found; the estimate for IL-4 and PIQ was somewhat increased, but the 95% confidence interval included the null value ( $P = 0.06$ ) (Table 3).

To explore potentially modifying effects of preterm or SGA status on associations between cytokine concentrations

**Table 1.** Basic Characteristics of the Study Population ( $n = 369$ ), Infant Growth Project, Jefferson County, Alabama, 1985–1988

	No. of Subjects	Total	% <sup>a</sup>	Mean (SD)
Infant characteristics				
Female gender	179	369	48.5	
Gestational age, weeks				38.2 (2.7)
Birth weight, g				3,031 (649)
Preterm birth (<37 weeks)	72	369	19.5	
Small-for-gestational-age <sup>b</sup> birth	75	369	20.3	
Race/ethnicity				
White	104	369	28.2	
Black/African-American	265	369	71.8	
Maternal characteristics				
Age, years				24.2 (4.3)
Maternal education				
Less than high school diploma	123	368	33.4	
High school diploma	126	368	34.2	
≥1 years of college	119	368	32.3	
PPVT-R score				73 (12.9)
Pregnancy characteristics				
Cesarean delivery	52	329	15.8	
Infection during pregnancy	61	335	18.2	
Antibiotics during labor	22	337	6.5	
Intrapartum fever >100.4°F	6	321	1.9	
Smoking during pregnancy	158	363	43.5	
Alcohol drinking during pregnancy	118	369	32.0	
Poverty <sup>c</sup>	226	322	70.2	
Home Screening Questionnaire score				36.8 (6.2)
Child IQ score <70				
Verbal IQ	47	369	12.7	84.2 (11.6)
Performance IQ	43	369	11.7	84.5 (13.4)

Abbreviations: IQ, intelligence quotient; PPVT-R, Peabody Picture Vocabulary Test–Revised; SD, standard deviation.

<sup>a</sup> Denominators vary because of missing values for different variables; percentages may not total 100 because of rounding.

<sup>b</sup> Below the 10th percentile of weight-for-age based on a fetal growth standard (34).

<sup>c</sup> Family income below \$9,600 per year.

and limitations in intellectual function, we conducted stratified analyses by preterm birth status (Table 4) and SGA birth status (Table 5). Among children who were born preterm (mean gestational age = 33.9 weeks; standard deviation, 2.3), a 1 log-unit increase in TNF- $\alpha$  was associated with a reduced odds ratio for a VIQ score less than 70 (adjusted odds ratio (OR) = 0.11, 95% confidence interval (CI): 0.01, 0.94), but not among term-born children (adjusted OR = 1.32, 95% CI: 0.65, 2.66) (Table 4). Among SGA-born children, 1 log-unit increase in TNF- $\alpha$  was associated with an elevated odds ratio for VIQ <70 (adjusted OR = 3.96, 95% CI: 0.99, 15.9), while no association with TNF- $\alpha$  was seen among those born non-SGA (Table 5). Estimates for IL-12p70 and PIQ remained significant among term and non-SGA births, while confidence

intervals widened for IFN- $\gamma$  (Tables 4 and 5). We also evaluated all models in Tables 3–5 without adjusting for birth weight, and the findings remained very similar (Web Tables 2–4).

Thirteen of the 72 preterm children were also SGA (18%). After excluding these children from the analysis, the inverse association between TNF- $\alpha$  concentrations and VIQ <70 (Table 4) was strengthened among preterm children (adjusted OR = 0.04, 95% CI: 0.002, 0.69). In addition, an inverse association for VIQ <70 and IL-12p70 concentrations was found (adjusted OR = 0.11, 95% CI: 0.01, 0.96). Among term SGA children, the association between VIQ and TNF- $\alpha$  was slightly weakened (adjusted OR = 3.18, 95% CI: 0.84, 12.1;  $P = 0.09$ ); for the relation of PIQ to IFN- $\gamma$  concentrations, the estimate also changed slightly (adjusted

**Table 2.** Geometric Mean Levels of Umbilical Cord Serum Cytokines According to Verbal and Performance Intelligence Quotient Scores ( $n = 369$ ), Infant Growth Project, Jefferson County, Alabama, 1985–1988

Cytokine	VIQ Score				PIQ Score			
	<70 ( $n = 47$ )		$\geq 70$ ( $n = 322$ )		<70 ( $n = 43$ )		$\geq 70$ ( $n = 326$ )	
	GM, pg/mL	SD	GM, pg/mL	SD	GM, pg/mL	SD	GM, pg/mL	SD
Interferon- $\gamma$	6.3	2.3	5.9	2.6	4.7*	2.1	6.1	2.6
Tumor necrosis factor- $\alpha$	8.8	1.6	8.7	1.6	8.6	1.9	8.7	1.6
Interleukin-12p70	3.8	1.5	4.0	1.7	3.4*	1.5	4.0	1.7
Interleukin-10	4.3	2.3	4.4	2.1	4.4	2.4	4.4	2.1
Interleukin-4	8.1	2.0	8.3	2.7	6.7	2.4	8.5	2.6

Abbreviations: GM, geometric mean; PIQ, performance intelligence quotient; SD, standard deviation; VIQ, verbal intelligence quotient.

\*  $P < 0.05$  (2-sided  $t$  test).

OR = 0.45, 95% CI: 0.18, 1.11;  $P = 0.08$ ), while the remaining findings were virtually unchanged.

## DISCUSSION

Our study found that increases in circulating concentrations of the proinflammatory T helper 1 cytokines IFN- $\gamma$  and IL-12p70 at birth were related to reduced odds of developing a low PIQ at age 5 years of approximately 30% and 50%, respectively. The association between low PIQ and IL-4, a T helper 2 cytokine, was of similar magnitude as that for IFN- $\gamma$  but with a wider confidence interval. For TNF- $\alpha$ , also a critical T helper 1 cytokine, 1 log-unit increase was protective against a low VIQ among preterm children but was associated with increased odds of low VIQ among SGA children. Our findings among SGA and preterm births support a differential role of TNF- $\alpha$  in the developing neurologic system (44, 45). Overall, our results suggest that certain inflammatory cytokines in umbilical cord blood may be markers of perinatal processes directly or indirectly involved in child intellectual development.

A body of research has suggested that severe infant neurologic illnesses, such as white matter lesions or cerebral palsy (1), are related to clinically severe prenatal infection, with potentially differential effects according to gestational age

and weight at birth (4–7). Investigators have concluded that cytokines might be mediators of underlying processes (4–7). However, most of those studies did not assess actual cytokine concentrations or IQ. Few studies have measured cytokines at birth and assessed cognitive development in children prospectively. In one earlier study conducted among very preterm infants born at 23–31 weeks of gestation, Andrews et al. (46) found no association between cord blood concentration of IL-6 or any maternal inflammatory marker, including severe chorioamnionitis, and IQ at 6 years of age. No other cytokines were assessed in that study. The findings with regard to maternal inflammatory markers are in line with ours, although we had few women with reported clinical markers of more severe inflammation such as chorioamnionitis ( $n = 4$ ) in our sample. We cannot directly compare the findings of Andrews et al. (46) with ours, since our study included few very preterm births (<32 weeks:  $n = 9$ ), and we could not assess IL-6, which is known to be an important regulator of neurogenesis in adults and in some neurodegenerative diseases (47) but has an unclear role in early neurodevelopment.

While there is a paucity of human data, evidence from animal and in vitro studies is starting to accumulate. Studies with mice have shown that IL-12p70, which is secreted from dendritic cells and stimulates IFN- $\gamma$  production in naive T cells, can induce de novo neurogenesis and remyelination. Moreover, mice treated with IL-12p70 showed significant

**Table 3.** Adjusted Odds Ratios<sup>a</sup> for Low (Score <70) Verbal (VIQ) and Performance (PIQ) Intelligence Quotient Scores (Reference Category:  $\geq 70$ ) and Adjusted Linear Regression Coefficients<sup>a</sup> for VIQ and PIQ as Continuous Variables at Age 5 Years in Relation to Umbilical Cord Serum Log Cytokine Concentrations (pg/mL) ( $n = 369$ ), Infant Growth Project, Jefferson County, Alabama, 1985–1988

Cytokine	VIQ Score <70		PIQ Score <70		VIQ (Continuous)		PIQ (Continuous)	
	OR	95% CI	OR	95% CI	$\beta$	95% CI	$\beta$	95% CI
Interferon- $\gamma$	1.03	0.74, 1.44	0.67*	0.46, 0.98	0.43	-0.81, 1.67	0.79	-0.68, 2.26
Tumor necrosis factor- $\alpha$	0.97	0.50, 1.86	0.81	0.41, 1.60	0.66	-1.76, 3.09	-0.53	-3.41, 2.34
Interleukin-12p70	0.73	0.38, 1.37	0.43*	0.21, 0.87	1.59	-0.70, 3.88	1.93	-0.77, 4.64
Interleukin-10	0.85	0.55, 1.31	0.90	0.59, 1.40	0.87	-0.63, 2.37	0.39	-1.39, 2.18
Interleukin-4	0.95	0.68, 1.32	0.71	0.49, 1.02	0.60	-0.61, 1.82	1.34	-0.08, 2.76

Abbreviations: CI, confidence interval; OR, odds ratio; PIQ, performance intelligence quotient; VIQ, verbal intelligence quotient.

\*  $P < 0.05$  (logistic regression; reference category:  $\geq 70$ ).

<sup>a</sup> Adjusted for birth weight, gender, and race/ethnicity; VIQ results were additionally adjusted for smoking during pregnancy (any).

**Table 4.** Adjusted Odds Ratios<sup>a</sup> for Low (Score <70) Verbal and Performance Intelligence Quotient Scores (Reference Category:  $\geq 70$ ) at Age 5 Years in Relation to Umbilical Cord Serum Log Cytokine Concentrations, by Preterm Birth Status ( $n = 369$ ), Infant Growth Project, Jefferson County, Alabama, 1985–1988

Cytokine	Preterm Birth ( $n = 72$ )				Term Birth ( $n = 297$ )			
	VIQ Score <70		PIQ Score <70		VIQ Score <70		PIQ Score <70	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Interferon- $\gamma$	1.08	0.51, 2.28	0.66	0.21, 2.12	1.04	0.71, 1.52	0.72	0.47, 1.09
Tumor necrosis factor- $\alpha$	0.11*	0.01, 0.94	0.05	0.001, 2.43	1.32	0.65, 2.66	1.09	0.55, 2.18
Interleukin-12p70	0.49	0.12, 2.04	0.84	0.10, 7.43	0.82	0.40, 1.66	0.43*	0.20, 0.94
Interleukin-10	0.53	0.18, 1.59	1.45	0.38, 5.54	0.94	0.60, 1.49	0.92	0.59, 1.44
Interleukin-4	0.91	0.40, 2.09	0.90	0.24, 3.27	0.96	0.67, 1.37	0.72	0.49, 1.06

Abbreviations: CI, confidence interval; OR, odds ratio; PIQ, performance intelligence quotient; VIQ, verbal intelligence quotient.

\*  $P < 0.05$  (logistic regression; reference category:  $\geq 70$ ).

<sup>a</sup> Adjusted for birth weight, gender, and race/ethnicity; VIQ results were additionally adjusted for smoking during pregnancy (any).

improvement in locomotor function (48). Experimental research also suggests that IFN- $\gamma$  plays a protective role by inducing neurogenesis, with positive effects on learning and memory (12, 27, 48). IFN- $\gamma$  was shown to promote neuronal progenitor cell differentiation in vitro, and it has been suggested that IFN- $\gamma$  activity can promote the early development of the central nervous system (49). Overall, the experimental evidence supports our findings of a possible protective role of IFN- $\gamma$  and IL-12p70 during neurologic development, possibly manifested in inductive and deductive reasoning as captured by the PIQ tests and which are processes thought to be largely related to biologic factors.

Another possible explanation can be that higher levels of IFN- $\gamma$  and IL-12 may be markers of a better developed perinatal immune system, which is indicative of a more “robust” immune system in childhood (better protection against viral and bacterial infections) with better intellectual development. Unfortunately, in this study we did not have information about the number of postnatal infections with which to evaluate this hypothesis.

Elevated TNF- $\alpha$  concentrations were protective against VIQ <70 in preterm children but were associated with

increased odds of VIQ <70 among SGA children. No association was observed with VIQ <70 among term or non-SGA-born children or between TNF- $\alpha$  and PIQ <70 in any subgroup. To our knowledge, no other investigation of TNF- $\alpha$  concentrations at birth among preterm or SGA births in relation to intellectual function has been conducted to date. In experimental studies, under immunologically unchallenged conditions, TNF- $\alpha$  has been shown to be essential for normal memory and learning in mice (50). TNF- $\alpha$  has been linked to both neurodevelopment, by promoting the generation of neuronal networks and cell survival (51), and neurodegeneration, by triggering apoptosis (52). In young mice, TNF- $\alpha$  deficiency was detrimental for spatial memory but had opposite effects on older mice (50). TNF- $\alpha$  promoted in a dose- and time-dependent manner cell differentiation of dopaminergic neurons exerting neurotoxic and neuroprotective effects, suggesting a time-dependent switch of the TNF- $\alpha$  response during neurodevelopment (44). In a child brain imaging study, Betjemann et al. (53) demonstrated relations between specific cognitive functions and neuronal structures. Because TNF- $\alpha$  apparently exerts its activity with certain neuronal structures (44), it is possible that it

**Table 5.** Adjusted Odds Ratios<sup>a</sup> for Low (Score <70) Verbal and Performance Intelligence Quotient Scores (Reference Category:  $\geq 70$ ) at Age 5 Years in Relation to Umbilical Cord Serum Log Cytokine Concentrations, by Small-for-Gestational-Age Status ( $n = 369$ ), Infant Growth Project, Jefferson County, Alabama, 1985–1988

Cytokine	SGA Birth ( $n = 75$ )				Non-SGA Birth ( $n = 294$ )			
	VIQ Score <70		PIQ Score <70		VIQ Score <70		PIQ Score <70	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Interferon- $\gamma$	0.90	0.46, 1.73	0.65	0.32, 1.31	1.13	0.75, 1.69	0.73	0.46, 1.17
Tumor necrosis factor- $\alpha$	3.96* <sup>b</sup>	0.99, 15.9	2.22	0.69, 7.12	0.53	0.23, 1.23	0.52	0.22, 1.23
Interleukin-12p70	1.10	0.36, 3.40	0.78	0.25, 2.41	0.62	0.28, 1.36	0.32*	0.13, 0.82
Interleukin-10	1.23	0.68, 2.22	1.22	0.68, 2.18	0.66	0.35, 1.23	0.75	0.40, 1.42
Interleukin-4	1.00	0.48, 2.07	0.92	0.45, 1.86	0.96	0.65, 1.41	0.67	0.43, 1.05

Abbreviations: CI, confidence interval; OR, odds ratio; PIQ, performance intelligence quotient; VIQ, verbal intelligence quotient.

\*  $P < 0.05$  (logistic regression; reference category:  $\geq 70$ ).

<sup>a</sup> Adjusted for birth weight, gender, and race/ethnicity; VIQ results were additionally adjusted for smoking during pregnancy (any).

<sup>b</sup> Borderline-significant ( $P = 0.052$ ).

interacts under challenging conditions (i.e., SGA or preterm birth) specifically with structures linked with VIQ. In a twin study, associations were seen between nutritional fetal growth restriction and reductions in VIQ, while no effect on PIQ was seen, indicating that neuronal areas that underlie verbal abilities are more vulnerable to suboptimal in utero growth conditions than those associated with PIQ (54). This is in line with our findings suggesting that elevated TNF- $\alpha$  can be a marker for increased risk of low VIQ among growth-restricted children. One explanation for the different direction of TNF- $\alpha$  effects in preterm birth may be the time-dependent and cell-specific nature of TNF- $\alpha$ -mediated processes; thus, effects involving the same structures may be expressed differently, conditional on gestational age (44, 50, 51).

We observed effects on the binary outcome of intellectual limitations defined a priori based on the guidelines of the American Association on Intellectual and Developmental Disabilities and as done in previous reports (46), rather than on the continuous IQ outcomes. This suggests that a 1 log-unit increase in levels of certain cytokines is associated with the odds of having a low VIQ or PIQ rather than with change in mean IQ measures. This may suggest the existence of a subgroup of children who are particularly susceptible to processes associated with changes in cytokine concentrations.

Our longitudinal study entailed several limitations. We could not measure actual central nervous system concentrations of the cytokines. However, a body of evidence supports the notion that proinflammatory peripheral cytokines pass and exert activity beyond the blood-brain barrier in utero (18, 22, 24, 45, 55, 56). Cytokine patterns have been shown to vary across lengths of gestation (58), as well as potentially by environmental determinants (58). Ideally, we would have had consecutive prenatal and postnatal child and maternal in utero peripheral and central nervous system measurements of cytokines with which to compare the effects of the cytokines at the different time points and in different compartments, as well as objective information on timing and severity of prenatal inflammation. Cotinine levels in umbilical cord blood were not measured, and secondhand exposure to tobacco smoke during pregnancy was not assessed. Our sample size was relatively small. Larger numbers in subgroups would have allowed more detailed analysis of a possible impact of inflammatory and noninflammatory preterm birth, as well as of maternal inflammatory illnesses.

Our study had a number of marked strengths, particularly the prospective nature of the study and the availability of detailed medical information, which allowed us to assess a large number of potentially confounding variables. Confounding is very unlikely to explain the results reported herein, as the final models controlled for all important confounders in this sample. Moreover, we can rule out observer and reporting bias, since the cytokine analysis was conducted blinded to the children's intellectual outcome status.

In summary, inflammatory cytokines are part of a complex cellular bidirectional communication network with responses related to synergistic or antagonistic actions of its various components. It is possible that the effects we observed are caused indirectly, via immune system response, or directly, by cytokine action at and beyond the fetal blood-brain barrier.

The results of this study may help identify at birth predictive markers of potentially susceptible subgroups. Further epidemiologic research closely integrating in utero molecular markers is needed to advance the understanding of complex mechanisms in early human neurologic and cognitive development.

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