

chamber, and plated onto enriched or selective agar media. Media for recovery of obligate anaerobes included pre-reduced Brucella base blood agar containing 5% sheep blood, hemin, and menadione (BMB, PML, Tuluatin, OR), and BMB containing 5% laked sheep blood, 100 mcg/ml of kanamycin, and 7.5 mcg/ml vancomycin (BKV) for *Bacteroides* and *Prevotella* species. Facultative organisms were isolated with tryptic soy base 5% sheep blood agar (BAP, PML, Tuluatin, OR), bile esculin azide agar for *Enterococci*, MacConkey's agar for *Enterobacteriaceae*, and Rogosa selective agar for lactobacilli and bifidobacteria.

Following incubation under appropriate atmospheric conditions and lengths of time, as recommended for the recovery of the specific groups of microorganisms, colonies were enumerated on the various selective media, and individual colony types were selected for identification by gram stain, based on colony morphology. All counts were recorded as \log_{10} CFU/gram dry weight sample. The lower limit of detection of the various organisms was $1.5 \log_{10}$ CFU/gram.

Statistical analysis

The distribution of cytokine levels was skewed, with a significant number of undetectable values; therefore, median cytokine levels are presented. Differences in the levels of cord blood cytokines between neonates born by vaginal delivery and those born by cesarean section were examined by nonparametric two-sample Wilcoxon tests. In complementary analyses adjusting for potential confounders, we estimated the effect of mode of delivery on cytokine secretion by CBMCs by stepwise linear regres-

sion. In these analyses, cytokine values were \log_{10} -transformed. In addition, we estimated the odds of having detectable cytokine levels at birth for children born by cesarean section compared with those born by vaginal delivery using stepwise logistic regression. In the final models, we included variables that were significant at $P < 0.05$ or that satisfied a change in estimate criterion ($\geq 10\%$) in the parameter estimate.

The following variables were considered for inclusion in the multivariate analysis: race/ethnicity, gender, gestational age, birth weight, birth length, Apgar score, maternal age, and maternal history of atopy (a physician's diagnosis of any of the following: asthma, eczema, hay fever, or allergy).

In exploratory analyses, we examined whether the maternal gut flora (a close correlate of the maternal vaginal flora) had different influences on neonatal cytokine production depending on mode of delivery. We calculated Spearman's correlation coefficients (r_s) for the number of microbes for specific bacterial species in maternal stool and cytokine levels in cord blood, first in all subjects and then after stratification by mode of delivery (vaginal vs. cesarean section). All analyses were performed with SAS version 8 (SAS Institute, Cary, NC).

Results

Population characteristics

Table 1 shows the characteristics of the 37 participating mother-child pairs. The mean age of participating women was 33.3 years (standard deviation [SD] = 6.1 years); approximately 60% of participating women had a history

Table 1: Characteristics of Maternal-Infant Pairs in Relation to Mode of Delivery

	Total (N = 37)	Vaginal Delivery (N = 22)	Cesarean Section (N = 15)	p-value
N (%)				
Race/Ethnicity				
White	25 (67.6)	14 (63.6)	11 (73.3)	0.83
Black	3 (8.1)	2 (9.1)	1 (6.7)	
Hispanic	8 (21.6)	5 (22.7)	3 (20.0)	
Others	1 (2.7)	1 (4.6)	0 (0)	
Gender				
Male	21 (56.8)	12 (54.5)	9 (60)	1.00
Female	16 (43.2)	10 (45.5)	6 (40)	
Maternal history of atopy*	22 (59.5)	14 (63.6)	8 (53.3)	0.53
Maternal smoking during pregnancy	2 (5.4)	1 (4.6)	1 (6.7)	1.00
NICU admission	1 (2.7)	0 (0)	1 (6.7)	0.41
Mean (range)				
Maternal age, years	33.3 (17.9–43.2)	31.9 (17.9–40.6)	35.3 (25.8–43.2)	0.08
Neonatal birth weight, kg	3.4 (2.2–4.4)	3.3 (2.2–4.3)	3.6 (3.0–4.4)	0.03
Neonatal gestational age, weeks	39.0 (36.0–41.0)	38.9 (36.0–41.0)	39.1 (37.0–41.0)	1.00
Neonatal birth length, cm	49.7 (44.0–53.0)	49.5 (44.0–53.0)	50.0 (46.0–53.0)	0.48
APGAR at 5 minutes	8.7 (1–10)	8.9 (8.0–10.0)	8.4 (1.0–10.0)	0.98

*Atopy = doctor diagnosed asthma, eczema, hay fever, or allergies