Table 3: Association between Cesarean Section and Cytokine Secretion by CBMCs

Outcome		Adjusted‡ β*	Adjusted‡ p-value
IL-13			
	Media	0.9	0.08
	Fel d 1	**1.56**	**0.007**
	Der f 1	**1.9**	**0.0006**
	PHA	**1.82**	**0.002**
	LPS	**1.38**	**0.01**
IFN-γ			
	Media	**1.24**	**0.02**
	Fel d 1	**1.23**	**0.01**
	Der f 1	0.90	0.07
	PHA	**1.27**	**0.03**
	LPS	0.76	0.17
IL-10			
	Media	-0.66	0.08
	Fel d 1	-0.51	0.42
	Der f 1	-0.36	0.60
	PHA	-0.11	0.88
	LPS	-0.19	0.70

*Change of one log10 pg/ml cytokine level in children born by cesarean section as compared to children born by vaginal delivery.
‡Foroutcome IL-13, model adjusted for maternal age, maternal atopy, neonatal birth weight, neonatal gestational age, and neonatal birth length.
For outcome IFN-γ, model adjusted for maternal age, maternal atopy, and neonatal birth weight.
For outcome IL-10, model adjusted for maternal age, neonatal gestational age, and neonatal birth weight.

the pathogenesis of atopy in children born by cesarean section [22-24].

The observed association between mode of delivery and neonatal immune responses may be explained by absent or reduced labor in children delivered by cesarean section. The process of labor may directly influence neonatal immune responses, thereby influencing cytokine secretion at birth. Although a relationship between labor and neonatal secretion of IL-13, IFN-γ, and IL-10 has not been shown, the stress of labor has been associated with decreased T lymphocytes and CD4+ helper T cells [25,26], and increased neutrophils [27,28], natural killer (NK) cells [26,28], TNF-α [29], and IL-6 [13,29] in cord blood. In contrast, cesarean section without labor has been associated with increased T lymphocytes and CD4+ helper T cells [25,26], decreased neutrophils [27,28], natural killer (NK) cells [26,28], TNF-α [29], and IL-6 [13,29] in neonates at birth.

Although labor itself may have important immunoregulatory effects on neonates [25-28] and thus partly explain our findings, it is also plausible that the observed neonatal cytokine profile in children born by cesarean section is due to their reduced contact with the maternal vaginal flora at birth. We measured the composition of the maternal gut flora, which is strongly correlated with that of the maternal vaginal flora.

Our findings with regard to bacterial species in the maternal intestinal flora and neonatal immune responses should be interpreted with caution because of small sample size and inability to control for confounders such as maternal diet. However, our preliminary results in children born by vaginal delivery are interesting and suggest the possibility that exposure to specific microbes in the maternal vaginal flora during passage through the birth canal influences neonatal immune responses. In particular, we found that gram-positive anaerobes and total anaerobes in maternal stool were associated with increased secretion of IL-10 by CBMCs, and that gram-negative anaerobes and gram-negative aerobes in maternal stool were associated with reduced secretion of IL-13 and IFN-γ by CBMCs.

The observed association between anaerobes in maternal stool and increased neonatal secretion of IL-10 by CBMCs (at 24 hours after stimulation with allergens) is consistent with results of experiments in murine models and in vitro studies in humans. Stimulation of cord blood lymphocytes with gram-negative bacteria for 24 hours (including anaerobes such as *Bacteroides* species) results in strong secretion of IL-10 [30]. In rodents, peritoneal cells produce IL-10 after stimulation with the CPC of *Bacteroides fragilis* [31]. More specifically, PSA from *B. fragilis* elicits IL-10 production from a population of murine Tregs beginning at 24 hours after stimulation [32]. In murine models, the PSA molecule of *B. fragilis* is presented to T cells by intestinal dendritic cells (DCs) residing at mucosal surfaces, which then activate CD4+T cells and elicit appropriate cytokine secretion resulting in a balanced Th1/Th2 immune response [33]. Moreover, DCs have been shown to mediate the secretion and activity of Tregs [34,35].

Administration of lactobacilli to atopic children has been associated with increased production of cytokines produced by Tregs (e.g., IL-10) [36,37]. It is thus plausible that early modulation of immune responses by specific bacteria (e.g., anaerobes) during passage of the neonate through the birth canal [38] results in upregulation of neonatal Tregs and/or direct downregulation of Th1 and Th2 immune responses. The effects of labor may further interact with those of the maternal gut flora on neonatal immune system development.

The observed association between gram-negative anaerobes and increased secretion of IL-13 (at baseline and after stimulation with *Fel d 1* and LPS) and IFN-γ (after stimulation with LPS) by CBMCs in children born by