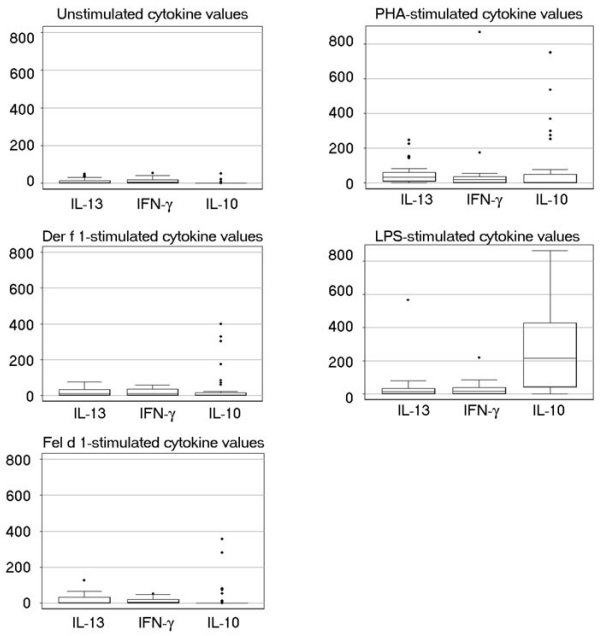

Figure 1
Secretion of IL-13, IFN-γ, and IL-10 cytokines by neonatal CBMCs. IFN-γ, IL-10, and IL-13 secretions were measured in unstimulated supernatants and supernatants after 24 hours' stimulation with allergens (*Der f 1* and *Fel d 1*), phytohemagglutinin (PHA), and lipopolysaccharide (LPS). The median is represented by the black bar. The upper and lower boundaries of the box represent the 25th to 75th percentiles of the data, respectively. Observations < 1.5 times the height of the box beyond either quartile are displayed within the whiskers. (•) represents outliers.

and with IFN-γ secretion by CBMCs at baseline and in response to allergens. There was no association between maternal atopy and neonatal levels of IL-10.

Our results for the logistic regression analyses of the relation between mode of delivery and detectable cytokine secretion in neonates were similar to those of the linear regression analyses shown above. For example, in multivariate logistic regression analyses, cesarean section was associated with increased odds of having detectable levels of IL-13 in cord blood (odds ratio [OR] = 26.0; 95% confidence interval [CI] = 2.0, 336.8) and IFN-γ (OR = 30.8; 95% CI = 1.7, 555.9) after stimulation with *Fel d 1*. Although not statistically significant, cesarean section was associated with reduced odds of having detectable IL-10 secretion after stimulation with *Fel d 1* (OR = 0.42; 95% CI = 0.06–2.87).

Maternal gut flora and cord blood cytokines by mode of delivery

We found no significant differences in the numbers of anaerobic or aerobic bacteria in maternal stool between women who delivered by cesarean section and those who delivered vaginally. There was no significant difference in the composition of the maternal gut flora of atopic and nonatopic women (data not shown).

In the analysis including all subjects (n = 37), total anaerobic bacteria in maternal stool were positively correlated with secretion of IL-10 by CBMCs after stimulation with *Fel d 1* (r_s = 0.44, p = 0.008) and *Der f 1* (r_s = 0.36, p = 0.03) allergens. In addition, gram-positive anaerobes (lactobacilli and bifidobacteria) were associated with increased IL-10 secretion in response to *Der f 1* (r_s = 0.37, p = 0.02), and gram-negative anaerobes (*Bacteroides* and *Prevotella*) were associated with increased IL-10 secretion in response to *Fel d 1* (r_s = 0.40, p = 0.02).

Table 4 shows the results of the analysis of the relation between the maternal gut flora and cytokine secretion by CBMCs after stratification by mode of delivery. Among children born by vaginal delivery, total anaerobes and gram-positive anaerobes in maternal stool were each associated with increased secretion of IL-10 by CBMCs after stimulation with *Fel d 1* and *Der f 1*. In contrast, gram-negative aerobes *(Enterobacteriaceae)* in maternal stool were negatively correlated with secretion of IL-13 (after stimulation with *Der f 1*) and IFN-γ (at baseline and after stimulation with antigens [*Fel d 1*, *Der f 1*, and LPS]).

Among children born by cesarean section, gram-negative anaerobes in maternal stool were associated with increased secretion of IL-13 by CBMCs at baseline and after stimulation with *Fel d 1* and LPS, and with increased secretion of IFN-γ in response to LPS stimulation (Table 4).

Discussion

To our knowledge, this is the first study to demonstrate an association between cesarean section and increased neonatal secretion of IL-13 and IFN-γ. This finding provides a potential immunologic basis for previous reports of an association between cesarean section and atopy or asthma [2-8], as elevation of IL-13 [9-11] and IFN-γ [11,12] at birth has been associated with asthma and atopy in childhood. In addition, there was a non-statistically significant trend for an inverse association between cesarean section and neonatal levels of IL-10 (a cytokine with inhibitory effects on the secretion of Th1 and Th2 cytokines in vivo) [19-21]. Although this finding should be further assessed in larger studies, it suggests that abnormal stimulation of mechanisms that downregulate both arms of the immune response (e.g., T regulatory cells [Tregs]) may influence