Perinatal Bacterial Exposure Contributes to IL-13 Aeroallergen Response

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Abstract

There is a high prevalence of aeroallergen sensitivity in asthmatic populations, and seroreactivity to aeroallergens early in infancy is associated with increased risk of developing asthma later in life. In addition to allergen sensitivity, asthma development has been associated with differential microbial exposure and infection in early life. We have previously shown that cord blood mononuclear cells respond to common aeroallergens (i.e., house dust mite [Der f1] and cockroach [Bla g2]) as assayed by lymphoproliferation and cytokine (IL-13 and IFN- γ) production. We hypothesized that there is a relationship between perinatal microbial exposure and response to specific aeroallergens. To test this hypothesis, we isolated DNA from cord blood serum samples with known lymphoproliferative and cytokine responses to Bla g2 and Der f1. Bacterial 16S ribosomal DNA amplicon libraries were generated and analyzed using high throughput sequencing of cord blood serum samples. In our analysis, we identified major compositional differences, including diversity and abundance of specific taxa, between groups whose IL-13 response to Der f1 and Bla g2 differed. We demonstrate a strong association between the ratio of *Acinetobacter* to Proteobacteria and IL-13

production and the probability of IL-13 production after allergen exposure. IL-13 concentrations in serum were also significantly correlated with the diversity of bacterial DNA. Together, these results underscore the relationship between immune responses to allergens and bacterial exposure during perinatal development.

Keywords: fetal blood; IL-13; allergen; *Acinetobacter*; asthma

Clinical Relevance

There is a high prevalence of aeroallergen sensitivity in asthmatic populations, and seroreactivity to aeroallergens early in infancy is associated with increased risk of developing asthma later in life. In addition to allergen sensitivity, asthma development has been associated with differential microbial exposure and infection in early life. These results underscore the relationship between immune responses to allergens and bacterial exposure during perinatal development.

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Atopic asthma is characterized by obstruction of air flow through the bronchial tree in response to specific aeroallergens, generally beginning early in life. Sensitization to common aeroallergens, such as those from the house dust mite (HDM) and cockroach, has been recognized as an important predictor of decreased pulmonary function and increased airway hyperresponsiveness (1, 2). Specifically, children sensitized to HDM allergens have an increased risk for respiratory infections, chronic wheeze, and the development of asthma and atopy that persist into adulthood (3–5). This sensitization is associated with higher levels of HDM allergen-specific IgE (5). An important relationship exists between altered immune responses to aeroallergens and the development of asthma and atopy in childhood.

Interestingly, many of these disordered immune responses, such as increased IgE levels, lymphoproliferation, and altered cytokine production in response to aeroallergen stimulation, are detected as early as the perinatal time period (6–9). Along with altered immune responses, differential environmental exposure to microbes and colonization of mucosal sites by microbes early in life have been linked to the development of asthma (10, 11). This has led to postulations that early-life interactions between hosts and microbes are essential for development of the normal immune system, and perturbations of these interactions can lead to disordered immune responses such as those seen in asthmatics (12).

Recent examinations of mucosal sites other than the airways, such as the gut, have shown that the differential growth of specific bacteria is related to asthma development (13). Additionally, environmental factors (e.g., breastfeeding) can influence bacterial colonization of mucosal surfaces early in life (14). Airway colonization by certain bacterial pathogens, such as *Streptococcus*, *Moraxella*, and *Haemophilus*, in early life can enhance susceptibility to both upper and lower respiratory infections from bacteria and virus (15). Along with altered immune responses to aeroallergens, individuals who are sensitized to specific allergens (e.g., HDM allergens) also show disordered humoral recognition of bacterial antigens (16). Thus, there is a relationship between bacterial colonization and host immune recognition, and differential recognition may be involved in allergen sensitization and asthma development.

In addition to the traditional sites of bacterial colonization, such as the gut and the upper airways, there have been reports of bacteria and bacterial nucleic acids at solid tissue sites and in the blood stream. With the use of traditional techniques for bacterial detection and identification (e.g., Gram's staining and culture-independent techniques), several studies have detected both bacteria and bacterial DNA within placental tissue, and correlated the detection of nucleic acids derived from different bacterial species with disease states, including urinary tract infections, preeclampsia, and preterm birth (17–20). In murine models, specific bacteria are capable of being enriched within the placental tissue (21). Along with potential fetal exposure to bacteria, many bacterial metabolites and cellular products appear capable of traversing the placental barrier (22, 23). Thus, exposure to microbial antigens and products may occur before birth and may influence the development of the immune system.

Along with localization within solid tissues, bacterial DNA has been reported to circulate in the blood stream of healthy individuals (24). The detection of bacterial DNA in blood does not appear to be limited only to adults, and can also be isolated from umbilical cord blood (25). The amount and composition of bacterial DNA have been linked to several disease states in both adults and newborns (26, 27); however, the relation of the bacterial DNA in circulation to development of the immune system remains largely unexplored. In this study, we investigated the role of bacteria-derived DNA in relation to the generation of lymphoproliferation and T helper cell 1 (Th1)/Th2 cytokine production by umbilical cord blood mononuclear cells (CBMCs) in response to aeroallergen stimulation. We demonstrate that several aspects of bacterial DNA, including diversity, taxonomic structure, and source, are significantly linked with the production of IL-13 by CBMCs stimulated with aeroallergens.

Materials and Methods

Study Population, Lymphoproliferation, *In Vitro* Cytokine Measurements, and Clustering

The current study was approved by the University of Illinois at Chicago IRB

(#2016–0326). Volunteers were recruited from the eastern Massachusetts general population. The exclusion criteria were multiple gestation, inability to answer questions in English, gestational age $\geq$ 22 weeks at recruitment, and plans to move away before delivery. The cohort profile was previously described by Oken and colleagues (28). Umbilical cord blood was obtained from consenting mothers and CBMCs were assessed for lymphoproliferation, IFN- γ , and IL-13 after stimulation with Der f1 (HDM) and Bla g2 (cockroach) allergens (6). The procedure for sample classification based on lymphoproliferation and cytokine response is outlined in Figure 1, and more detailed information about the procedure can be found in the online supplement.

DNA Isolation

DNA was extracted from 1 ml of cord blood serum using a QIAmp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All extractions were performed under sterile conditions. Each extraction was recorded for the lot number on the kit and time of extraction, and tested for any effect in our analysis. No effect was observed for the lot number or date of extraction. To control for any possibility of bacterial DNA contamination introduced in our procedure, we used 1 ml of molecular-grade water in our extraction protocol as a negative control. Our negative control showed both a minimum five-cycle time difference as assessed by qPCR from any sample and did not yield a 16S ribosomal DNA (rDNA) library. The DNA concentration and base pair size were quantified in all samples using Qubit dsDNA High Sensitivity Dye (Invitrogen, Carlsbad, CA) and on a 2100 Bioanalyzer using a High Sensitivity DNA Kit (Agilent, Santa Clara, CA).

16S rDNA Amplicon Sequencing and Analysis

The V1–V3 regions of the 16S rDNA gene were amplified and the DNA libraries were sequenced on Illumina's MiSeq platform. For operational taxonomic unit (OTU) annotation, we used the QIIME pipeline (29). Additional information regarding the methods used can be found in the online supplement. Sample data, OTU counts, and taxonomy were imported and stored in the R statistical environment using the R package phyloSeq (30). OTU counts were normalized using

Figure 1. Schematic of sample classification. Samples were clustered by stimulation index to Bla g2 or Der f1, and only samples that clustered as low- or high-stimulation index were included in the final analysis. The cytokine response to Bla g2 and Der f1 was characterized as response or no response. PHA, phytohemagglutinin.

DESeq blind normalization (31).

To assess for β diversity, we performed a constrained correspondence analysis, and calculated Fisher's α and rank-abundance distributions using the R package *vegan* (32).

Multiplex Cytokine and Chemokine Assay

Serum was centrifuged at $10,000 \times g$ for 10 minutes to clear any cellular debris. The serum was then assessed for concentrations of IFN- γ , -4, -5, and -13 using Bio-Plex Pro Human assays (Bio-Rad). Concentrations were determined using the Bio-Plex 200 system.

Statistics

The Mann-Whitney U test was used in all two-group comparisons unless otherwise stated. Permutational multivariate ANOVA was used to assess group differences in β diversity. Linear regression was used to correlate IL-13 and IFN- γ concentrations after stimulation with Der f1 or Bla g2 with bacterial family abundances, and P is the probability that the slope was nonzero. $P < 0.05$ was considered significant in all situations. Linear and logistic regression was used to correlate IL-13 and IFN- γ concentrations after stimulation with media, phytohemagglutinin, Der f1, or Bla g2 with the log ratio of Moraxellaceae to Proteobacteria.

Data Accessibility

The metagenomic sequence data for the samples have been uploaded to MG-RAST (<http://metagenomics.anl.gov/>) and are available under Project ID: 16926.

Results

Bacterial 16S rDNA Fragments Circulate in Perinatal Umbilical Cord Blood and Are Derived from Multiple Taxonomic Families

Consistent with previous reports (33), we isolated 10–100 ng of fragmented DNA from human serum (Figure E1A in the online supplement). We were able to quantitate the 16S rDNA fragments (base pairs 926–1062) from all samples using universal bacterial 16S rDNA primers by qPCR assay, and all samples amplified with a minimum five-cycle difference compared with negative controls (Figure E1A) (34). Along with prior reports of bacterial DNA in adult peripheral blood (24), our results indicate that human umbilical cord blood contains bacterial DNA.

To assess the bacterial source of these fragments, we amplified the V1–V3 region (~500–700 bp) of the bacterial 16S rDNA from the isolated DNA. We successfully amplified DNA libraries of 16S rDNA amplicons in 27 out of 38 samples (71%). We speculate that we were not able to generate libraries in all of our samples due to the larger amplicon size compared with our qPCR reactions (~600 versus 140 bp) and because we started with DNA that contained fragments smaller than 600 bp (Figure E1B). From each library we generated an average of ~560,000 paired-end reads per sample. These reads were assigned to 846 unique OTUs. The overwhelming majority (>99%) of reads assigned to OTUs were derived from four core phyla that were detected in a minimum of one read in $\geq 60\%$ of all

samples: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Figure 2A). Notably, we observed that a large portion of the bacterial families were sparsely distributed (minimum of one read in < 30% of all samples) and that only 13 families were identified as the core bacterial families in our samples (Figure 2B). Taken together, these results indicate that bacterial DNA is present in the circulation during the perinatal time period and derived from multiple bacterial sources.

The IL-13 Cytokine Response to Common Aeroallergens Is Related to the Taxonomic Structure and Diversity of Bacterial DNA

We previously found that peripheral and CBMCs exposed to aeroallergens (cockroach [Bla g2] and HDM [Der f1]) produce differential lymphoproliferative and Th1/Th2 cytokine responses, and that these responses are related to risk factors for the development of asthma (6, 8). Here, we assessed whether these allergen-specific responses correspond to unique signatures of bacterial DNA in the circulation. First, to categorize the samples, each sample with a known lymphoproliferative response was clustered by stimulation index to Bla g2 and Der f1 (Figures E2A and E2B). Similarly, to categorize the cytokine response, samples were grouped by the type of cytokine produced in response to Bla g2 and Der F1, and whether they produced detectable IL-13 or IFN- γ .

To identify differences in taxonomic structure, sample-wise distances were calculated using weighted unique fraction metric (UniFrac) distances (Figure E3A),

Figure 2. Phylogenetics of bacterial DNA in umbilical cord blood. (A) Unrooted phylogenetic tree displaying all operational taxonomic units (OTUs). The line weighting is proportional to the percentage of samples in which one read was mapped to an OTU. Four core phylum (Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes) are displayed by color (green, blue, red, and purple, respectively). (B) Dendrogram of core bacterial families, colored by phylum, with size proportional to the normalized counts in each sample.

and group medoids were compared. Only the presence (responder [R]) or absence (nonresponder [NR]) of IL-13 production after allergen stimulation identified groups

with significantly different medoids (Figure 3A). There were no significant differences between groups of low- and high-stimulation index or groups of IFN- γ

responders and nonresponders, although there was a trend ($P < 0.1$) of different medoids categorizing IFN- γ . Thus, our data indicate that there is a relationship between

Figure 3. Bacterial DNA diversity and composition differ in the IL-13 response to aeroallergens. (A) Principal component (PC) analysis plot (first and second components) of weighted UniFrac distances between IL-13 nonresponders (NR, solid square) and responders (R, open circle) as defined by detectable IL-13 after Bla g2 or Der f1 exposure. Significance ($P < 0.05$) was determined by permutational multivariate ANOVA. (B) α -Diversity measurements between IL-13 nonresponders and responders, displayed by Fisher's α of individual samples. (C) Relative abundances of core phyla and sparse phyla (other) between IL-13 nonresponders (NR) and responders (R). (D) Normalized counts of Proteobacteria between IL-13 nonresponders (NR) and responders (R). Groups are shown as median with interquartile range. Counts were $\log_{10}$ transformed with a pseudocount of one added for visualization of samples with zero counts. * $P < 0.05$, Mann-Whitney U test. PCoA, principal coordinates analysis.

allergen-specific IL-13 responses and the composition of bacterial DNA. Based on this observation, we focused our remaining analysis on the specific relationship between the IL-13 response and bacterial DNA.

We next examined the α diversity of each sample by calculating Fisher's α parameter for each sample. This parameter was selected because the majority of samples displayed logarithmic distributions on rank-abundance curves (Figure E3B), which fits Fisher's model. When we compared IL-13 nonresponders with responders, we observed a significant increase in Fisher's α parameter in responders (Figure 3B). By assessing sample-wise UniFrac distances, we showed that changes in family abundance (weighted UniFrac) were present when IL-13 nonresponders were compared with responders. Additionally, we found that IL-13 responders had an increased evenness of OTU abundances, and that nonresponders had significantly increased representation by fewer OTUs. These results indicate that Th2 responses to common aeroallergens are related to the source of bacterial DNA, and not simply to the presence of microbial DNA.

The Common Aeroallergen IL-13 Response Is Related to Specific Bacterial Phylum and Family Abundances

To identify taxonomic differences in the composition of bacterial DNA between nonresponders and responders, we compared each group's normalized counts of the four core phylum. Only the bacterial abundance of Proteobacteria was increased in nonresponders (Figures 3C and 3D). The additional core bacterial phylum, Actinobacteria, Bacteroidetes, and Firmicutes, showed no significant differences between groups (Figure 3D).

Interestingly, when we examined the abundances of core bacterial families, we found three families that had significantly increased abundances in responders: Micrococcaceae, Moraxellaceae, and Rhodobacteraceae, two of which are of the phylum Proteobacteria (Figure 4A). Although there was an overall increase in the abundance of Proteobacteria-derived DNA in samples with no response to aeroallergens, samples with Th2-skewed responses to aeroallergens had increased DNA derived from specific families of Proteobacteria, notably the human pathogens in the family Moraxellaceae. Additionally, there was a trend ($P = 0.08$)

toward increased abundance of sequences derived from Corynebacteriaceae in responders (Figure 4A). All other core bacterial families displayed no change in abundance between groups (Figure E4).

Circulating Bacterial DNA in the Perinatal Time Period Correlates with IL-13 Production in Response to Common Aeroallergens

To assess whether differentially abundant families of bacterial DNA in the perinatal circulation are predictive of the quantity of IL-13, we correlated core bacterial families that had increased abundances in responders with the amount of IL-13 that was produced after Der f1 or Bla g2 stimulation. For two of the increased bacterial families in the responders, Corynebacteriaceae and Moraxellaceae, the DNA abundance significantly correlated with increased IL-13 production after Der f1 stimulation (Figure 4B). Interestingly, both of these bacterial families (Corynebacteriaceae and Moraxellaceae) are known to be colonizers and pathogens of the skin, oropharynx, and upper and lower respiratory tracts (15). There were no significant correlations between any of the four bacterial families tested with Bla g2-induced IL-13 production.

Figure 4. Specific bacterial family abundances are increased with the IL-13 response to aeroallergens. (A) Normalized counts of bacterial families, with significant differences between IL-13 nonresponders and responders. Groups are shown as median with interquartile range. Counts were log₁₀ transformed with a pseudocount of one added for visualization of samples with zero counts. $*P < 0.05$, Mann-Whitney U test. (B) Scatter plots of normalized counts of bacterial families with corresponding cord blood mononuclear cell IL-13 production after allergen stimulation. Only samples with an IL-13 response to Der f1 (solid square) or Bla g2 (open square) are displayed. Linear regression of IL-13 responders is shown as a solid line, and 95% confidence intervals are shown as dashed lines for Der f1 (black) or Bla g2 (gray) response. $*P < 0.05$.

There was also a trend ($P = 0.08$) toward increasing IL-13 production after Der f1 stimulation in Rhodobacteraceae, and no significant correlation with Micrococcaceae (Figure 4B). Additionally, there were no significant correlations with any of these bacterial families and the production of IFN- γ (Figure E5). This is consistent with previous reports that increasing IL-13 production in CBMCs does not alter IFN- γ production after allergen stimulation (6).

The Moraxellaceae (Comprised of *Acinetobacter*) to Proteobacteria Ratio Is Predictive of the IL-13 Response to Aeroallergens

To combine our findings into a predictive model of response, we compared the Moraxellaceae/Proteobacteria ratio with the quantity and presence of an IL-13 response. Moraxellaceae is a family within Proteobacteria, a diverse phylum of gram-negative bacteria, and this ratio represents the percentage of Proteobacteria that is derived from the family Moraxellaceae. The Moraxellaceae/Proteobacteria ratio was chosen to reconcile the fact that Proteobacteria was increased in IL-13 nonresponders, yet Moraxellaceae was increased in abundance in IL-13 responders and its abundance correlated positively with IL-13 production.

We found that the Moraxellaceae/Proteobacteria ratio was positively correlated with IL-13 production after Der f1 or Bla g2 exposure (Figure 5A). Additionally, an increasing Moraxellaceae/Proteobacteria ratio was significantly associated with a greater probability of an IL-13 response after Der f1 exposure, and there was a trend ($P < 0.1$) toward greater probability after Bla g2 exposure (Figure 5B). These associations were specific to aeroallergen exposure and the IL-13 response. We observed no significant associations when we assessed for IFN- γ response or IL-13 production after exposure to media or phytohemagglutinin (Tables 1 and 2). We observed that there were two genera that composed the reads annotated in Moraxellaceae: *Acinetobacter* and *Enhydrobacter* (Figure 6). In IL-13 nonresponders and responders, 7 of 12 and 9 of 9 samples with detectable Moraxellaceae, respectively, had $> 50\%$ of the Moraxellaceae reads assigned to *Acinetobacter* ($P < 0.05$, Fisher's exact test).

Figure 5. The Moraxellaceae/Proteobacteria ratio is predictive of the IL-13 response to aeroallergens. (A) Linear regression of the Moraxellaceae/Proteobacteria ratio in all samples versus the IL-13 response is shown as a solid line, and 95% confidence intervals are shown as dashed lines for response to Der f1 (black) and Bla g2 (gray). (B) Logistic regression of the Moraxellaceae/Proteobacteria ratio in all samples versus the probability of an IL-13 response is shown as a solid line, and 95% confidence intervals are shown as dashed lines for Der f1 (black) or Bla g2 (gray) response.

Serum DNA Microbial Diversity Is Associated with Serum IL-13 Cytokine Concentrations

To assess the effect of the serum DNA microbial composition on *in vivo* Th1 and Th2 cytokine concentrations, we correlated the Moraxellaceae/Proteobacteria ratio with IL-4, -5, -13, and IFN- γ . We did not observe any significant associations between the cytokines and the Moraxellaceae/Proteobacteria ratio (Table E1). To determine whether there is an association between diversity and cytokine concentrations, we correlated IL-4, -5, -13, and IFN- γ with Fisher's α parameter. Similar to our previous findings with allergen-stimulated CBMCs, IL-13 concentrations were significantly associated with Fisher's α parameter (Figure 7; Table E2). There was also a trend ($P = 0.08$) toward an association between IL-4 and

Fisher's α parameter. There were no significant associations between IL-5 or IFN- γ and Fisher's α parameter.

Discussion

We have demonstrated that a diverse array of bacterial DNA is present within the perinatal circulation. The bacterial DNA is derived from four main phyla: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria. Diversity of the taxonomy from which these sequences were derived was related to whether or not CBMCs produced IL-13 in response to Der f1 or Bla g2. Additionally, the ratio of *Acinetobacter* to proteobacterial DNA is predictive of IL-13 responses to common aeroallergens. Along with *in vitro* IL-13 production in response to cytokines, IL-13

Table 1. Association of the Moraxellaceae/Proteobacteria Ratio with Cytokine Production as Determined by Univariate Linear Regression

		β	Confidence Interval (95%)	R^2	P
IFN- γ	Media	-3.483	-9.155 to 2.189	0.060	0.217
	PHA	1.5e3	-1038 to 4162	0.058	0.228
	Bla g2	46.91	-41.61 to 135.4	0.045	0.285
	Der f1	298.8	-199.7 to 797.3	0.057	0.228
IL-13	Media	6.509	-2.797 to 15.82	0.077	0.162
	PHA	-102.1	-1350 to 1146	0.001	0.868
	Bla g2	7.667	1.085 to 14.25	0.187	0.024
	Der f1	19.96	5.595 to 34.33	0.247	0.008

Definition of abbreviation: PHA, phytohemagglutinin.

Table 2. Association of the Moraxellaceae/Proteobacteria Ratio with the Probability of Cytokine Response as Determined by Univariate Logistic Regression

		β	SE	P
IFN- γ	Media	0.320	1.619	0.843
	PHA	3.240	3.038	0.286
	Bla g2	0.821	1.894	0.665
	Der f1	0.116	1.898	0.951
IL-13	Media	2.362	1.892	0.212
	PHA	< 0.001	> 1.0e5	1.000
	Bla g2	13.57	8.093	0.093
	Der f1	5.550	2.503	0.026

For definition of abbreviation, see Table 1.

derived from serum significantly correlated with the diversity of bacterial DNA in serum.

IL-13, an IgE-inducing cytokine, is regulated by environmental factors such as microbial exposure at later time periods in childhood (35). Even as early as the perinatal time period, exposure to microorganisms and their products may be important for generating Th2 responses to foreign antigens, which is important for class switching to IgE. Allergy to HDM aeroallergens is found in up to 85% of individuals with asthma (4). In addition to the high prevalence of IgE due to HDM allergens in asthmatics, children with higher levels of IgE due to HDM allergens are more likely to be susceptible to respiratory viruses and the development of wheezing (5). Asthmatics have a differential humoral response to bacterial antigens, specifically those that are induced by Th2 cytokines (e.g., IgE), as well as IgG1 and

IgG4 (16, 36, 37). We speculate that detection of bacterial DNA may represent exposure to specific bacteria.

Increased HDM-induced IL-13 production was strongly correlated with the abundance of *Acinetobacter* relative to Proteobacteria within a sample (Figure 5). Interestingly, in a study by Fyhrquist and colleagues (38) involving nonatopic individuals (age 13–20 yr), exposure to *Acinetobacter* was associated with increased production of IL-10 and Th1 cytokines. In the same study, it was observed in those with high IgE levels to inhaled allergens that exposure and colonization with *Acinetobacter* was not associated with IL-10 and immunoregulatory gene expression. In atopic individuals, *Acinetobacter* may be inversely correlated with IL-10 production and expression (38, 39). Our model of the IL-13 response to aeroallergens predicts that an increasing representation of *Acinetobacter* within Proteobacteria

enhances the likelihood and quantity of IL-13 production in response to Der f1 or Bla g2. Thus, both the quantity of *Acinetobacter* and the diversity of Proteobacteria are important for determining atopic-like responses to common aeroallergens. Our observation is similar to a previous finding that in the skin microbiome, *Acinetobacter* inversely correlates with IL-10 production, and there is reduced diversity within Gammaproteobacteria in atopic individuals (39).

There were several limitations to our study that may be addressed in future work. Future studies could include analyses of additional sites of interest (e.g., maternal serum and placental tissue) that could provide insight into origins of the bacterial DNA that we detected. Given our study's sample size ($n = 27$), we chose to focus our analyses on the most abundant bacterial families and phyla. Future work with a larger sample size may allow for further analysis of rarer bacterial families. Although multiple cytokines may be of interest in determining T cell phenotypes related to asthma, due to the limited sample volume, we focused on clinically relevant Th2 (IL-4, -5, and -13) and Th1 (IFN- γ) responses. Although we did not directly detect bacteria, the sequencing of DNA for indirect detection has been used in other situations, such as detection of fetal and tumor DNA (40–42), and bacterial DNA in serum may represent exposure to those bacteria at other sites on the body. Additionally, the detection of specific bacterial DNA within the circulation may be associated with specific disease states (27).

Figure 6. Moraxellaceae are comprised mainly of *Acinetobacter* in cord blood serum. The dendrogram displays all OTUs assigned to Moraxellaceae. *Left:* the shape designates the genus, and if a species could be identified, it is listed. *Right:* bar graph displaying the percentage of reads assigned to Moraxellaceae composed of each genus.

Figure 7. Linear regression of Fisher's α diversity using serum IL-13 (pg/mL) as a linear predictor.

We demonstrate that the composition and diversity of bacterial DNA in perinatal cord blood serum correlates with the production of a Th2 cytokine during

allergen challenge to CBMCs and in serum. Notably, we observed stronger associations between bacterial DNA and IL-13 with an *in vitro* allergen challenge than in serum. This may be due to the many potential influences that T cells would be under *in vivo*. The possible implications for health outcomes remain to be determined. For example, we have shown a decrease in diversity and increase in Proteobacteria in nonresponders (Figures 2 and 3).

There are differences in diversity and the abundance of Proteobacteria in bronchoalveolar lavages between asthmatics and healthy individuals (43–45). Our study adds to the growing picture that there is a relationship between how individuals respond to

allergens and how they are colonized and respond to their own microbiota. Specific immune responses to allergens may be predictive of a potential colonization of bacteria later in life and susceptibility to asthma development. The importance of early-life exposure and interaction with bacteria as early predictors of asthma and atopy later in life merits further investigation. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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