



Letter

Clostridia in the gut and onset of atopic dermatitis via eosinophilic inflammation

The balance of commensal and pathogenic microbiota in the intestine plays an important role in immune system development through the establishment of gastrointestinal epithelial barriers and immune system homeostasis.¹ Alterations to this balance may cause systemic chronic inflammation and may lead to the development of allergic diseases.¹ Studies on the association between gut microbiota in early life and atopic dermatitis (AD) led to inconclusive results with a poor mechanistic understanding.^{2–6}

We investigated the diversity and composition of gut microbiota in 12 infants with AD and 12 healthy infants at 6 months of age. We elucidated the association among gut microbiota, age at onset of AD, and percentage of blood eosinophils to identify the underlying mechanisms of gut microbiota in the development of AD after the control of confounding factors. All the children were born by vaginal delivery and had no history of antibiotic and/or probiotic use during the first 6 months of life. The percentage of blood eosinophils, total serum IgE levels, and severity of AD using the Scoring Atopic Dermatitis (SCORAD) index were simultaneously assessed at the time of fecal collection. The 16S ribosomal RNA gene sequencing approach was used to amplify for the V1 to V3 regions (eSupplement).

Diversity of gut microbiota was increased in infants with AD compared with healthy infants without statistical significance. The richness score (Chao1, operational taxonomic units) and mean (SD) of relative abundance of bacilli at 6 months were significantly increased in infants with AD compared with healthy infants ($P = .030$; $P = .013$; 13.83% [4.91%] vs 3.13 [0.74%], $q = 0.0528$, $P = .0081$, respectively).

The relative abundance of *Clostridia* from infants with AD correlated with age at AD onset (Table 1) and inversely correlated with percentage of blood eosinophils. Although the relative abundance of bacilli was not significantly correlated with age at AD onset, it correlated with the percentage of blood eosinophils. There was no significant correlation between the relative abundance of *Escherichia coli* (*E coli*) and age at AD onset. However, the percentage of blood eosinophils weakly correlated with the relative abundance of *E coli*. None of the gut microbiota was associated with the SCORAD index and total serum IgE levels in infants with AD or total participants (data not shown).

We identified that no significant differences in the diversity but significant differences in the richness of gut microbiota between infants with AD and healthy infants and specific gut microbiota are

associated with eosinophilic inflammation but not total serum IgE levels. These results suggest clues for the unestablished connection between gut microbiota and development of AD.

Studies on the diversity of gut microbiota in patients with AD have been inconclusive to date.^{2,4} One study reported that infants with IgE-associated AD had a lower diversity of total microbiota at 1 month of age,⁴ whereas another found no association between total fecal bacterial profiles and AD development.² Alterations in the balance of gut microbiota rather than diversity may be the driving force of AD development through immunomodulatory effects.¹

There have been diverse studies on the compositional differences in gut microbiota in patients with AD to identify its role in the development of AD.^{2–5,7} *Clostridia* are associated with the development of AD, although another study found no such association.^{5,7} We found that a higher abundance of gut *Clostridia* at 6 months of age correlated with later development of AD and was inversely associated with percentage of blood eosinophils. Several microbiota belonging to *Clostridia* are known to produce propionic acid and other short-chain fatty acids.⁸ Propionate modifies the function of dendritic cells, preventing the differentiation of naive T cells into T_H2 cells.⁹ Short-chain fatty acids decrease allergic inflammation through the modulation of cell activity, resulting from inhibition of histone deacetylase activity.¹⁰ These previous studies support our findings of a negative association between the relative abundance of *Clostridia* and blood eosinophil and delayed development of AD.

Bacilli include *Lactobacillus*, *Streptococcus*, and *Staphylococcus*, some of which are used as probiotics for the prevention or treatment of AD. We found a weak association between bacilli and blood eosinophil percentage, which suggests that the balance of bacilli subclasses might play a role in systemic eosinophilic inflammation. More in-depth studies are needed to identify the underlying mechanism that links bacilli and AD development.

E coli in the gut was associated with the increased risk of AD.^{2,7} However, studies on the role of *E coli* in the intestine are lacking. One of the plausible hypotheses is that lipopolysaccharide originated from *E coli* might play a role in the regulation of the immune system through the intestinal epithelial cells.¹ We found a positive association between the relative abundance of *E coli* and percentage of blood eosinophils, suggesting that *E coli* may be linked with AD via eosinophilic inflammation. Eosinophils play a critical role in the development of AD and regulation of immune homeostasis and microbiota profiles in the intestine.¹¹

Although our analyses were limited by small sample size and a single fecal collection time point, we were able to characterize the association between components of gut microbiota and blood eosinophils and age at onset of AD. Although we did not completely control the infants' diet, no significant difference was found in the feeding style. Nevertheless, our study has its strength in that we

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Table 1

Association Among Relative Abundance of Gut Microbiota, Age at Onset of AD, and Percentage of Blood Eosinophils

Relative Abundance	Age at Onset of AD		Percentage of Blood Eosinophils	
	β	P Value ^a	β	P Value ^a
<i>Clostridia</i>	17.26	.002	–2.29	.03
Bacilli	1.54	.74	0.95	.07
<i>Escherichia coli</i>	2.70	.59	0.79	.09

Abbreviation: AD, atopic dermatitis.

^aPermutation test.

excluded several confounding factors, which can affect the gut microbiota. We used statistical methods to address the interactions among specific gut microbiota. These data will help in the understanding of the role of gut microbiota in the development of allergic diseases, including AD.

In summary, no significant differences were found in the diversity of gut microbiota between vaginally delivered infants with AD and healthy infants. High relative abundance of *Clostridia* correlates with later development of AD and is inversely related to percentage of blood eosinophils. The relative abundance of bacilli and *E coli* weakly correlated with blood eosinophil percentage. These findings suggest that gut microbiota may be linked to the development and/or onset of AD via eosinophilic inflammation.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2016.04.019>.

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eSupplement*Methods**Sample Collection, Preparation, and Analysis*

Collected fecal specimens were immediately frozen and stored at -80°C . DNA was extracted from the fecal samples using a Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, California). Amplification and sequencing were performed using a 454 GS FLX Titanium sequencing system (Roche, Branford, Connecticut). Lower-quality sequence reads (≤ 300 base pairs or a mean quality score ≤ 25) were removed to control quality. Representative sequences in each cluster were assigned their taxonomic positions by BLAST searches using the EzTaxone database. Chimeric sequences were removed using UCHIME and diversity indices were analyzed with the Mothur program.

Statistical Analysis

Read counts were assumed to follow the Poisson distribution, but the goodness-of-fit test revealed overdispersion problems. Therefore, a negative binomial distribution with log as the link function was assumed, and total read counts were incorporated as an offset to adjust differences between samples. P values based on the likelihood ratio test may be invalid if the sample size is not large enough; thus, permutation P values from 10,000 replicates were calculated. False discovery rates were estimated with q values for multiple testing control. The relative abundance of gut microbiota regressed with age at onset of AD, total serum IgE levels, and percentage of blood eosinophils. P values for the regression coefficients were calculated with permutation based on 5,000 replicates. All the statistical analyses were performed with R Statistical Software. $P < .05$ was considered statistically significant. In some cases, $P < .10$ but $\geq .05$ was considered clinically significant.