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How do Microbiota Influence the Development and Natural History of Eczema and Food Allergy?

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WHAT FACTORS PREDISPOSE INFANTS TOWARD DEVELOPING ECZEMA AND FOOD ALLERGIES?

Eczema (syn. "atopic eczema" or "atopic dermatitis") affects at least one-fifth of the pediatric population in industrialized nations, often arises in early infancy and raises the risk of developing subsequent sensitization, food allergy and asthma. Pedigree studies of families carrying filaggrin loss-of-function mutations demonstrate a semi-dominant inheritance pattern for eczema.[1] Filaggrin loss-of-function variants impair keratinocyte differentiation and reduce the ability of the skin to retain water, leading to poor skin barrier function and dry skin.[2] However, it is not known what instigates the cutaneous inflammation associated with eczema, although bacterial pathogens and changes in the diversity of the cutaneous microbiota may play a role. *Staphylococcus aureus* is, for instance, commonly found on the skin of eczema sufferers, especially with more severe disease. However, it remains uncertain whether bacterial dysbiosis (microbial imbalance associated with reduced diversity and prominence of pathogenic strains) on the skin plays a causal role in the development of eczema and disease flares, or whether the observed expansion of *S. aureus* and reduction in bacterial diversity are primarily an epiphenomenon resulting from an impaired and inflamed skin barrier.

Of the infants who develop eczema, a significant proportion become sensitized to food and go on to develop clinical food allergy.[3] Food allergy affects around 6% of young children across the UK. From birth, the gastrointestinal tract must learn to distinguish between food allergens and antigens associated with pathogens. Germ-free animal models that are reared without contact with microorganisms have dramatically reduced gut-associated lymphoid tissue and a strong tendency toward developing food allergies. Allergies arise after the food allergen has been sampled by dendritic cells and presented to the adaptive immune system, promoting the development of B cells that produce specific IgE which cross link on the allergen's surface. Gut-associated lymphoid tissue evolved in order to distinguish between symbiotic commensals resident in the gastrointestinal tract and potential pathogenic microorganisms. The complex interplay between the host's adaptive immune system, its commensal microbiota and the immunological recognition of food allergens demonstrates how resident microorganisms of the cutaneous and gastrointestinal compartments may influence the host and its immunological predisposition. Dysbiosis, may therefore abrogate oral tolerance allowing allergic responses to develop.

The "hygiene hypothesis" was born out of the observation that the risk of hay fever and eczema is inversely associated with the number of siblings.[4] At the time, David Strachan postulated that children living in larger families were protected from developing eczema and allergies by greater exposure to pathogens. Conventional bacterial culture-based work has suggested that commensal bacteria and pathogens may influence the development of eczema. For instance, fecal samples from Estonian and Swedish children with eczema or allergic sensitization demonstrated a significantly higher prevalence of *S. aureus* with less frequent enterococci and bifidobacteria colonization during infancy.[5] Recently, studies using molecular methods such as real-time polymerase chain reaction on fecal samples collected at 1 month of age have shown that colonization with *Escherichia coli* is associated with a greater risk of

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TABLE 1. Prospective Studies Measuring Diversity Indices With Later Assessment of Eczema or Sensitization

First author, Year, Journal	Cohort, Country	Number of Cases, Controls	Clinical Case Definition	Microbiologic Technique Used	Age at Sampling	Main Results
Abrahamsson, 2012, *J Allergy Clin Immunol*	Probiotic trial, Sweden	20, 20	Eczema associated with allergic sensitization until 2 yrs	V3-4 16S parallel sequencing	1 mo	Reduced Shannon diversity among cases ($P < 0.004$)
Azad, 2015, *Clin Exp Allergy*	CHILD cohort, Canada	12, 152	Food sensitized at 12 mos	V4 16S parallel sequencing	3 mos	Median Chao1 index: 25.0 vs. 28.0, $P = 0.02$. No significant difference in Shannon diversity detected
Wang, 2008, *J Allergy Clin Immunol*	Allergy Flora cohort, Sweden/Italy/UK	15, 20	Eczema associated with allergic sensitization by 18 mos	Terminal restriction fragment length polymorphism	7 d	Reduced number of TRFLP peaks amongst cases (7.0) than controls (9.5; $P = 0.03$)
Forno, 2008, *Clin Mol Allergy*	Recruited from Brigham & Women's Hospital, Boston, US	9, 21	Eczema at 6 mos	Denaturating gradient gel electrophoresis	1 and 4 mos	Cases demonstrated a lower Shannon index both at 1 ($P = 0.01$) and 4 mo of age ($P = 0.02$)
Ismail, 2012, *Pediatr Allergy Immunol*	Probiotic trial, Australia	Eczema; 33, 65 Sensitization; 20, 62	Eczema (UK Working Party criteria) and allergic sensitization	Terminal restriction fragment length polymorphism	7 d	Eczema cases demonstrated lower faecal microbial diversity vs. healthy (13.1 mean peaks vs. 15.5, $P = 0.003$ using restriction enzyme Alu1; 14.7 mean peaks vs. 17.2, $P = 0.03$ using Sau96I), but no difference with respect to skin sensitization
Bisgaard, 2011, *J Allergy Clin Immunol*	COPSAC, Denmark	Eczema; 127, 253 Sensitization prevalence not reported	Hanifin and Rajka criteria for eczema at visits up to 6 yrs Any sensitization measured by serum IgE	Denaturating gradient gel electrophoresis	1 and 12 mos	No relationship between faecal diversity and eczema. Diversity at 1 and 12 mo was inversely related to allergic sensitization (serum-specific IgE $P < 0.003$; skin prick test $P < 0.017$) at any of 0.5, 1.5, 4 and 6 yrs

eczema at 2 years, and that *Clostridia difficile* colonization in the gut is more common among children with eczema throughout the first 6 years of life (Dutch Child, Parent and Health: Lifestyle and Genetic Constitution (KOALA) birth cohort).[6,7]

With the advent of pyrosequencing technology, which is able to identify around 80% more bacterial strains than conventional culture-based methods, a much greater complexity and diversity of gut and skin microbiota has been demonstrated. Partly through this novel technology, the hygiene hypothesis has metamorphosed into the "biodiversity hypothesis," which proposes that the diversity of the gut and skin microbiota are of greater importance than identifying individual bacterial strains. It is reasoned that microbiota diversity aids the development of a regulatory T cell, cytokine and complement network, which protects against autoimmune conditions, allergies and eczema.[8]

We therefore conducted a systematic online literature search in Medline to identify and appraise the current evidence for the role of the skin and gut microbiota in the development and natural history of eczema and food allergy. We included both studies that looked at the risk of future eczema and food allergy development, as well as research that studied the association between the skin and gut microbiota in established disease.

PROSPECTIVE STUDIES RELATING EARLY FECAL MICROBIOTA CHARACTERISTICS TO THE DEVELOPMENT OF ECZEMA

The diversity or richness of infants' gut bacterial constituents has been reported in 6 birth cohorts, and each supports inverse relationships with eczema and/or allergic disease (Table 1). Two studies utilized 16S ribosomal rRNA gene pyrosequencing of fecal DNA among cases and controls selected from larger cohorts. For instance, Abrahamsson et al[9] found that 20 children with eczema and allergic sensitization selected at 18 months had a less diverse fecal microbiota at 1 month of age compared with healthy controls. In a Canadian study, children with lower gut microbiota richness at 3 months of age were more likely to develop food sensitization by their first birthday when compared to controls.[10] The remaining 4 studies used gel-based techniques to assess the diversity of the fecal microbiota, and 3 of these (the European collaborative Allergy Flora project, Australian and American studies) also reported reduced diversity among cases of eczema.[11–13] Bisgaard et al[14] found a reduced diversity among fecal samples supplied from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) cohort at 1 month of age among children developing any allergic sensitization up to 6 years of age, although there were no associations with eczema.

Studies have also investigated specific gut microbiota constituents before the development of allergic disease. For instance, the Dutch KOALA birth cohort found a greater prevalence of *E. coli* and *Clostridium difficile* in those who later developed eczema.[7] Infants carrying *Lactobacillus paracasei* were significantly less likely to develop eczema by 2 years. Other groups have assessed the "maturity" of the gut microbiota by comparing constituents. For instance, a population-based cohort from Winnipeg in Canada (mentioned above) found greater Enterobacteriaceae and lesser Bacteroidaceae at 3 months among the 12 infants who were found to be food sensitized at 1 year compared with controls.[10] The authors went on to rationalize that their Enterobacteriaceae/Bacteroidaceae ratio may represent gut microbiota maturity, and found that each quartile increase in Enterobacteriaceae/Bacteroidaceae ratio was associated with a 2-fold increase in risk of food sensitization. Abrahamsson et al[9] (mentioned previously) sequenced fecal samples passed at 1 week, 1 month and 1 year of age from 20 cases with both eczema and sensitization (diagnosed by either skin prick testing or serum-specific IgE) by 2 years, and compared these with controls who were participating in a probiotic

trial in Sweden. They observed that the samples passed at 1 month of age by cases who later developed eczema had a lower diversity of fecal microbiota, and also yielded greater proportions of obligate anaerobes, such as the *Bacteroidetes* phylum and *Bacteroides* genera. Similarly, eczema cases and controls selected from the placebo arm of a probiotic trial in Singapore showed a higher abundance of Enterobacteriaceae and *Clostridium perfringens* by fluorescence in situ hybridization combined with flow cytometry in those who developed eczema in the first 2 years life.

Thus, reduced gut microbial diversity in early life appears consistently associated with an increased risk of developing eczema, with the Dutch and Singaporean studies also reporting greater prevalence of Clostridia among cases with eczema, and Canadian, Swedish and Singaporean studies highlighting the relative abundance of Enterobacteriaceae. However, the picture is less clear with regards to Bacteroides genera, because the Swedish cohort found a greater abundance of *Bacteroidetes* phylum and *Bacteroides* genera among cases of eczema with sensitization, while the Canadian study appears to infer that increased relative abundance of Bacteroidaceae is associated with later protection from food sensitization.

CROSS-SECTIONAL STUDIES ASSESSING FECAL MICROBIOTA CHARACTERISTICS AMONG CHILDREN WITH ESTABLISHED ECZEMA AND MILK ALLERGY

While fecal samples from young infants have demonstrated lesser diversity of fecal microbiota among infants who later develop eczema and/or food sensitization, cross-sectional studies among participants with established disease report a less consistent picture. The largest cross-sectional study assessed 226 American children with established milk allergy and compared the diversity of their fecal microbiota according to whether their milk allergy resolved by 8 years. Of those children who enrolled between 3 and 6 months of age, fecal samples from milk allergic children enrolling between 3 and 6 months of age demonstrated a greater diversity and enrichment of *Clostridia* and *Firmicutes* phyla if they grew out of their milk allergy by 8 years.[15] The next largest cross-sectional study recruited 90 children and adults with eczema from outpatient clinics in South Korea, as well as 42 healthy controls and found no difference in overall gut microbiota diversity.[16]However, greater abundance of *Faecalibacterium prausnitzii* and reduced short-chain fatty acids were detected among patients with eczema less than 1 year of age. Metagenomic shotgun sequencing was consequently undertaken on a small subsample, which suggested greater functional responsiveness toward oxidative stress and handling of a major mucin component, N-acetylgalactosamine, among patients with eczema.[16]

Three other studies assessed gut microbiota characteristics associated with food allergy outcomes, with a recent pyrosequencing study from Naples reporting an *increase* in fecal diversity among 19 infants aged 1 to 12 months of age with IgE-mediated cow's milk allergy when compared with age-matched controls (Shannon indices for milk allergic infants 2.6 ± 0.4 vs. controls 1.7 ± 0.8, $P < 0.001$).[17] Another study investigated patients presenting to a hospital in Taiwan and found less fecal bacterial diversity among food sensitized children from 6 to 24 months of age when compared with those from 22 controls.[18] The last series investigated both IgE- and non-IgE-mediated cow's milk allergy versus healthy infants and reported no difference in fecal bacterial diversity.[19]

The current evidence relating fecal microbiota characteristics to allergic disease often conflates eczema, sensitization and clinical allergic disease. None of these studies assessed differences in dietary patterns or incorporated such data into their analyses. The studies investigating how gut microbiota characteristics may be associated with the development of eczema do not also consider the possibility that the skin microbiota may have a potential role.

ECZEMA AND THE SKIN MICROBIOTA

Topographic microclimates of human skin determine the ecological niches for occupying microbiota. Skin microbiota occupying sebaceous moist areas are, for instance, different in constituents and less diverse than those inhabiting dry extensor surfaces. The harmony of these communities may be pivotal in determining whether eczema arises, with mouse models suggesting that bacterial dysbiosis may cause eczematous dermatitis.[18] For instance, animal models lacking *Adam17* in *Sox-9*-expressing tissues demonstrate 2 waves of skin microbiota disturbance before eczema arises. First, *Corynebacterium bovis* proliferates and then *Staphylococcus aureus* becomes more abundant as the eczematous lesions develop. Intriguingly, treating these models with antibiotics prevented the majority of eczema, and also resulted in improved skin barrier function (lower transepidermal water loss) and lower total IgE levels, as well as a somewhat paradoxical increase in bacterial diversity of the skin.

PROSPECTIVE STUDIES RELATING CUTANEOUS MICROBIOTA CHARACTERISTICS TO THE DEVELOPMENT OF ECZEMA

Prospective cohort studies have failed to convincingly demonstrate that the early emergence of *S. aureus* on the skin is associated with the instigation of eczema. The COPSAC cohort found no relationship between *S. aureus* culture from nasal or perineal sites at either 1 month or 1 year and the later development of eczema up to 3 years of age.[19] While another study reported that *S. aureus* growth from nasal swabs at 6 months was associated with an increased eczema risk during the second year of life, a large proportion of these children already had already developed eczema by the time they were swabbed at 6 months.[20]

CROSS-SECTIONAL STUDIES ASSESSING SKIN MICROBIOTA CHARACTERISTICS AMONG CHILDREN WITH ESTABLISHED ECZEMA

Numerous culture-based studies support the clinical link between *S. aureus* burden and eczema severity. Greater skin barrier disruption, as measured by transepidermal water loss, is correlated with greater *S. aureus* colonisation.[21] Staphylococcal peptides and their superantigens have also been implicated in driving eczematous inflammation. For instance, patch testing adults with Staphylococcal enterotoxin B (SEB) induces an accumulation of T cells expressing SEB-reactive T-cell receptors, even in those without eczema.[22]

Recently, sequencing of skin microbiota from children with eczema has demonstrated that *Staphylococcus epidermidis* may also be involved in eczema pathogenesis. Eczema flares were not only associated with a significant fall in skin microbiota diversity but also a parallel increase in abundance of both *S. aureus* and *Staphylococcus epidermidis*. In addition, topical anti-inflammatory and antimicrobial treatment in the run up to the flare was associated with *reduced* abundance in *S. aureus* and a restoration of diversity, suggesting that eczema treatment can prevent the change in bacterial diversity associated with a disease flare.[23] Interestingly, the same team examined skin microbiota from patients with monogenic immunodeficiency states that are associated with eczematous rashes, including STAT3-deficient hyper-IgE and Wiskott-Aldrich syndromes. Markers of eczema severity were positively correlated with a higher prevalence of *S. aureus* and Corynebacterium and there was also greater diversity of opportunistic fungi and

less anatomical specificity in the context of immunodeficiency.

However, none of these studies have been able to compare subjects' skin microbiota with samples recovered before the inception of eczema, making it impossible examine the sequence of events between changes in the skin microbiota and eczema onset.

CAN WE LINK BOTH GUT AND SKIN DYSBIOSIS WITH THE DEVELOPMENT OF ECZEMA?

Perturbations of both the skin and gastrointestinal microbiota have been associated with eczema. We may hypothesize whether cross-talk between these environments may predispose toward the development or persistence of eczema. Our overview demonstrates that reduced gut microbiota diversity during early infancy is most consistently associated with the later development of eczema and allergic disease. By contrast, broad microbial assays such as pyrosequencing have demonstrated dysbiosis of the skin microbiota only among patients with established eczema so far. Nonetheless, it is likely that the interplay between physical properties of the local skin barrier and its occupying microbiota may raise the risk of developing eczema and, in turn, promote cutaneous mechanisms of atopic sensitization. Evidence suggests that the gut microbiome has a role in promoting greater tolerance to both dietary and environmental allergens, and perhaps dysbiosis and impaired gut microbiota diversity during infancy is related to failed allergen tolerance. Gut dysbiosis may facilitate the process of atopic sensitization, whether driven by sensitization through the skin, respiratory tract or elsewhere. This "double hit" of inflammatory skin microbiota with impaired allergen tolerance from gut dysbiosis may significantly increase infants' risk of developing eczema, sensitization and resulting allergic disease through childhood as supported by the evidence outlined in this review.

CHALLENGES TO INSPIRE FURTHER DEVELOPMENT

The current literature is small and hampered by methodological diversity and has failed to take important confounding factors into account, such as infant diet, hygiene practices and antibiotic prescribing. The studies described here are often observational and opportunistic. Birth cohorts often tended to select "more atopic" children as cases through conflating eczema, sensitization and clinical challenge-proven allergy in the hope of finding greater microbiome differences compared with healthy groups. Opportunistic clinic-based selection of participants will result in further bias. To identify microbiota changes associated with the emergence of eczema, well-phenotyped birth cohorts are needed with long-term follow-up. Rather than focusing on 1 body compartment alone, it will be important to study the skin, gut and perhaps even the oral cavity and respiratory tract together to allow a more holistic understanding of how our body microbiome interacts with the immune system and how it influences the development and natural history of eczema and food allergy (Table 1).

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