

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

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Introduction: what factors predispose infants towards 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 *Staphylococcus 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 gastro-intestinal tract must learn to distinguish between food allergens and antigens associated with pathogens. Germ free animal models who are reared without contact with micro-organisms have dramatically reduced gut-associated lymphoid tissue (GALT) and a strong tendency towards 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 which produce specific IgE which cross link on the allergen's surface. However GALT has evolved to distinguish between symbiotic commensals resident in the gastro-intestinal tract and potential pathogenic micro-organisms. We can therefore see how resident micro-organisms of the cutaneous and gastro-intestinal compartments influence the host and its immunological response as a symbiotic relationship. Dysbiosis, however, may not adequately promote oral tolerance and allergic responses may 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.^{4,4} 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 as well as 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 *Staphylococcus aureus* with less frequent enterococci and bifidobacteria colonization during infancy.⁵ More recently, studies using molecular methods such as real-time PCR on fecal samples collected at one month of age have shown that colonization with *Escherichia coli* is associated with a greater risk of eczema at 2 years, and that *Clostridia difficile* colonization in the gut is commoner among children with eczema throughout the first 6 years of life (Dutch 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 and that this 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 six 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 amongst cases and controls selected from larger cohorts. For instance, Abrahamsson *et al.* 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 to healthy controls.⁹ In a Canadian study, food sensitized one year olds carried significantly lower overall gut microbiota richness at 3 months of age when compared to controls.¹⁰ The remaining four studies used gel based techniques to assess the diversity of the fecal microbiota, and three of these (the European collaborative Allergy Flora project, Australian and American studies) also reported reduced diversity amongst cases of eczema.^{11,12,13} Bisgaard *et al.* found a reduced diversity amongst fecal samples supplied from the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) cohort at one month of age

amongst children developing any allergic sensitization up to 6 years of age, although there was no associations with eczema.¹⁴

Amongst studies conducted in infancy that looked for specific gut microbiota constituents, the Dutch KOALA birth cohort found a greater prevalence of *Escherichia coli* and *Clostridium difficile* in those who later developed eczema.⁷ Infants carrying *L. 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 amongst the 12 infants who were found to be food sensitized at one year compared to 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 twofold increase in risk of food sensitization. Abrahamsson *et al.* (mentioned above) sequenced fecal samples passed at one week, one month and one year of age from 20 cases with both eczema and sensitization (diagnosed by either skin prick testing or serum specific-IgE) by two years, and compared these to controls who were participating in a probiotic trial in Sweden. They observed that the samples passed by cases with eczema had a lower diversity of total fecal microbiota at one month of age, and also yielded greater proportions of obligate anaerobes, such as the *Bacteroidetes* phylum and *Bacteroides* genera.⁹ 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 (FISH) 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 amongst 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, since the Swedish cohort found a greater abundance of *Bacteroidetes* phylum and *Bacteroides* genera amongst cases of eczema with sensitization, whilst the Canadian study appears to infer that increased relative abundance of Bacteroidaceae is associated with later protection from food sensitization.

Cross-sectional studies assessing faecal microbiota characteristics amongst children with established eczema and milk allergy

Whilst fecal samples from young infants have demonstrated lesser diversity of fecal microbiota amongst infants who later develop eczema and/or food sensitization, cross-sectional studies amongst 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 amongst patients with eczema under one year

of age. Metagenomic shotgun sequencing was consequently undertaken on a small subsample, which suggested greater functional responsiveness towards oxidative stress and handling of a major mucin component, N-acetylgalactosamine, amongst 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 amongst 19 infants aged 1 to 12 months of age with IgE-mediated cow's milk allergy when compared to age-matched controls (Shannon indices for milk allergic infants 2.6 ± 0.4 versus 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 amongst food sensitized children from 6 to 24 months of age when compared to 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 two waves of skin microbiota disturbance before eczema arises. Firstly, *Corynebacterium bovis* expands 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 *Staphylococcus aureus* on the skin is associated with the instigation of eczema. The COPSAC cohort found no relationship between *Staphylococcus aureus* culture from nasal or perineal sites at either one month or one year and the later development of eczema up to three years of age.¹⁹ Whilst another study reported that *Staphylococcus 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 amongst children with established eczema

Numerous culture-based studies support the clinical link between *Staphylococcus aureus* burden and eczema severity. Greater skin barrier disruption, as measured by transepidermal water loss, is correlated with greater *Staphylococcus 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

More 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 *Staphylococcus aureus* and *Staphylococcus epidermidis*. In addition, topical anti-inflammatory and anti-microbial treatment in the run up to the flare was associated with *reduced* abundance in *Staphylococcus 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 *Staphylococcus 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 as well as disease flares.

Can we link both gut and skin dysbiosis with the development of eczema?

Perturbations of both the skin and gastro-intestinal microbiota have been associated with eczema. We may hypothesize whether cross-talk between these environments may predispose towards the

development or persistence of eczema. Our overview demonstrates that data describing alteration of the gastro-intestinal milieu is most robust before young infants first develop signs of eczema and allergic disease. By contrast, broad microbial assays such as pyrosequencing have demonstrated dysbiosis of the skin microbiota only amongst 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 to 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 one 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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Table 1. Overview of 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, Journal of Allergy and Clinical Immunology	Probiotic trial, Sweden	20, 20	Eczema associated with allergic sensitization until 2 years	V3-4 16S parallel sequencing	1 month	Reduced Shannon diversity amongst cases ($p < 0.004$)
Azad, 2015, Clinical Experimental Allergy	CHILD cohort, Canada	12, 152	Food sensitized at 12 months	V4 16S parallel sequencing	3 months	Median Chao1 index: 25.0 vs. 28.0, $P = 0.02$. No significant difference in Shannon diversity detected.
Wang, 2008, Journal of Allergy and Clinical Immunology	Allergy Flora cohort, Sweden / Italy / UK	15, 20	Eczema associated with allergic sensitization by 18 months	Terminal restriction fragment length polymorphism	7 days	Reduced number of TRFLP peaks amongst cases (7.0) than controls (9.5; $p = 0.03$)
Forno, 2008, Clinical & Molecular Allergy	Recruited from Brigham & Women's Hospital, Boston, USA	9, 21	Eczema at 6 months	Denaturing gradient gel electrophoresis	1 month and 4 months	Cases demonstrated a lower Shannon index both at 1 ($p = 0.01$) and 4 months of age ($p = 0.02$).
Ismail, 2012,	Probiotic	Eczema; 33,	Eczema	Terminal	7 days	Eczema

Pediatric Allergy and Immunology	trial, Australia	65 Sensitization; 20, 62	(UK Working Party criteria) and allergic sensitization	restriction fragment length polymorphism		cases demonstrated lower faecal microbial diversity versus 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, Journal of Allergy and Clinical Immunology	Copenhagen Prospective Study on Asthma in Childhood (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	Denaturing gradient gel electrophoresis	1 month and 12 months	No relationship between faecal diversity and eczema. Diversity at one and twelve months 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 years
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