The gastrointestinal microbiota and allergic disease

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The human body hosts innumerable and diverse microbes that outnumber human cells approximately 10-fold, the bulk of which reside in the gut.[1,2] Although most gut bacteria cannot be cultured,[3] metagenomic analyses estimate that there are approximately 4,000 different species, principally, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*, with minor fractions of other phyla.[4] This microbiota influences human health from birth to old age.[1] Emerging evidence suggests that besides conferring resistance to certain pathogenic infections and diseases, a homeostatic gut microbiota contributes to regulating body temperature, immune responses and hormones, nutrient breakdown, absorption and vitamin synthesis, and drug metabolism.[5]

The prenatal fetus is thought to be 'sterile'. Intestinal microbial colonization begins immediately after birth via vaginal delivery, inoculated by exposure to microorganisms that populate the birth canal, rapidly followed by others from the external environment. *Bifidobacteria* predominate over the first year,[6] their numbers increasing as populations of *Enterobacteriaceae* and *Bacteroides* decline and other groups gradually increase.[7] However, the healthy equilibrium of the gut microbiota can be disturbed in several ways. For example, delivery mode profoundly influences the microbial disposition; compared to infants delivered vaginally, those born by cesarean section have significantly less abundant fecal *Bifidobacteria* from 3–12 months (p =0.003), and a significant excess of *Lactobacilli-Enterococci* (p =0.002).[8] Gut microbe composition and quantities also change after weaning from breast milk, and with advancing age. Throughout life, lifestyle (eg, diet, sun exposure, probiotics), and individual health status (eg, obesity, antibiotic usage) play important roles in maintaining or disrupting microbial homeostasis.[5,9]

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> ***"Greater understanding of the microbiome provides a new focus for disease prevention and treatment."***
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The 'microflora' hypothesis

Emerging data support the 'microflora hypothesis', that increased incidence of allergic airway disease may result, at least in part, from disruption of normal gut microbiota-mediated immunological tolerance.[10] Studies in mice show that commensal bacteria confer important immunoprotective effects, while their disruption (dysbiosis) can contribute to susceptibility to chronic disease.[11] For example, tolerance to an oral ovalbumin challenge in germ-free mice was restored by reconstituting their intestinal microbiota with *Bifidobacteria*.[12] Compared to germ-free controls, mice with commensal bacteria also show attenuated allergic airway inflammation (a model of asthma) when exposed to ovalbumin,[13] while those with altered endogenous microbiota when first exposed to allergen would develop allergic airway responses.[10]

Dysbiosis has been linked with diverse human disorders, including: inflammatory bowel disease; colon cancer; irritable bowel syndrome; gastric ulcers; nonalcoholic fatty liver disease; hypertension; obesity and metabolic syndromes; mood and behavior modifications through hormone signaling; and diverse allergic diseases.[5,10] In a comparative study of the relationship between gut microbial disposition and allergy among infants from Estonia and Sweden, those who developed atopic dermatitis had smaller populations of *Enterococci* during the first month of life, and *Bifidobacteria* and *Bacteroides* during their first year, than non-allergic controls (p <0.05). In contrast, allergic infants harbored greater numbers of *Clostridia* at 3 months and *Staphylococcus aureus* at 6 months (p <0.05).[14] In Singapore, 3-year-olds with eczema had lower bifidobacterial stool counts than those without,[15] and eczema in cesarean-delivered

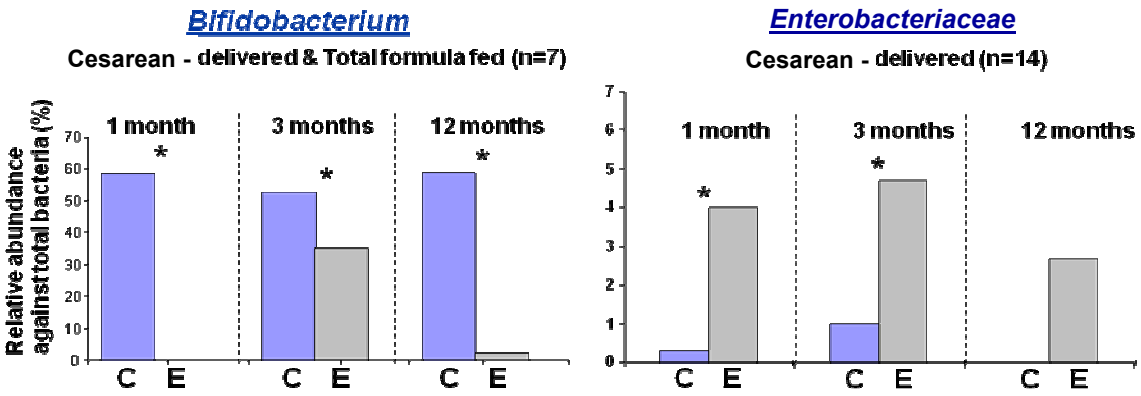

Figure 1. Association of eczema with reduced abundances of *Bifidobacterium* and higher abundances of *Enterobacteriaceae* (C, without eczema; E, with eczema)

Data source: Hong P-Y, Lee BW, Aw M, Shek L, Yap G, Chua KY, et al. Comparative analysis of fecal microbiota in infants with and without eczema. PLoS ONE 2010;5:e9964.

infants at 1, 3 and 12 months was similarly associated with reduced abundance of *Bifidobacteria* and higher abundance of *Enterobacteriaceae* (Figure 1).[16] Other studies have shown that eczema may be associated with not only the disposition, but also the overall diversity of gut bacteria during the immediate postnatal period. In a study of the correlation between infant gut microbiota and the development of atopy, Shannon-Wiener and Simpson indices of bacterial diversity were significantly lower in 1-week-old infants who had atopic eczema at 18 months versus healthy controls.[17] Subsequent analysis based on 16S rDNA sequencing corroborated the association of low intestinal microbial diversity during the first month of life with subsequent atopic eczema.[18]

Intervention with probiotics

Evidence that altering the gut microbiota may affect human health has driven rapid growth in the commercial development of probiotic supplements – live microorganisms believed to confer health benefits when consumed in adequate quantities. These are now widely marketed in numerous products, including infant feeds; however, investigations of probiotic dietary supplementation have failed to confirm definitive benefits, with equivocal and even contradictory results.

In a double-blind, randomized, placebo-controlled trial, *Lactobacillus GG* given 4 weeks prenatally to mothers and to their offspring until 6 months, halved the cumulative incidence of eczema at age 2 years among high-risk children with a family history of atopic allergic disease.[19] A similar supplementation regimen with four bacterial strains including *Lactobacilli* and *Bifidobacteria*, also reduced eczema incidence at 2 years, particularly atopic eczema.[20] Neither study showed general protection against allergic diseases besides eczema, or allergen sensitization. Other investigators have also noted more pronounced probiotic effects on immunoglobulin E (IgE) mediated disease. In a study of supplementation with *Lactobacillus reuteri*, there was no preventive effect against any potentially allergic diseases, including eczema; however treated infants did have less IgE-associated eczema at 2 years than controls.[21] Similarly, perinatal supplementation of a probiotic mixture to high-risk mothers and their infants had no protective effect against eczema, food allergy, allergic rhinitis, or asthma at 5 years, but the incidence of IgE-associated allergic disease was a significantly lower among cesarean-born children who received probiotics than those who did not.[22]

Efficacy in preventing eczema has been shown to vary between probiotics and inconsistently between studies. *Lactobacillus rhamnosus* given pre- and post-natally to mothers and their infants reduced the risk of eczema at 2 years compared to placebo, whereas *Bifidobacteria animalis subsp. lactis* did not.[23] However, *L. rhamnosus* and *Bifidobacterium longum* supplementation prevented neither eczema nor allergen sensitization, at 1 year among formula-fed Asian infants at risk of allergic disease.[24] Although a meta-analysis confirmed that probiotics reduce infant eczema, it highlighted the inconsistent

data and concluded that there is insufficient evidence to recommend adding probiotics to infant feeds to prevent allergic disease.[25] Given these uncertainties, further work is warranted to understand the importance of timing, dose and the organisms used, in probiotic supplementation.

Probiotics may also have undesirable effects. For example, *Lactobacillus acidophilus* supplementation for the first 6 months of life failed to reduce the risk of atopic dermatitis in high-risk infants and actually increased their risk of allergen sensitization at 12 months.[26] Likewise, *Lactobacillus GG* supplementation following the same protocol as Kalliomäki,[19] did not reduce the incidence or severity of atopic dermatitis, but was associated with increased frequency of recurrent wheezing bronchitis.[27] There are rare reports of nosocomial microbial infections associated with probiotic therapy.[28,29] Furthermore, probiotic products are not governed by the rigorous quality and safety standards that apply to medicines[30] and it is possible that contamination with cow milk protein may provoke allergic reactions in susceptible children.[31]

Notwithstanding shortcomings in the evidence that belie much of the extravagant marketing hype, it is unlikely that enthusiasm for probiotics will be a fleeting health fad. Rather, greater understanding of the microbiome provides a new focus for disease prevention and treatment in which foods may play a central part.

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