Perspective, Opinion, Commentary

Manipulating the Microbiome: What is Known, What is Unknown?

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We are outnumbered: there are more microbes in and on our bodies than our own host cells. The microbiome is a complex system that varies between individuals and, like, fingerprints, is unique. Colonization of newborns may occur even before birth via hematogenous spread of microbes from the mother's oral cavity and gastrointestinal tract into the intrauterine cavity. Thereafter and throughout life, gut and skin integrity play an important role in the health of the microbiome. There are multiple pathways to skin and gut dysbiosis, but the possibility of effective therapies remains promising.

Abbreviations:
- AD = atopic dermatitis
- CoNS = coagulase-negative staphylococci
- SCORAD = scoring atopic dermatitis
- SA = *staphylococcus aureus*

INTRODUCTION

We are outnumbered: there are more microbes in and on our bodies than our own host cells. While that ratio may be closer to 1:1 than prior estimates, there is no doubt that the human body is inhabited by large communities of bacteria, fungi, and viruses.[1] The term "microbiota" represents the ecological community of symbiotic, commensal, and pathogenic microorganisms themselves, while—technically speaking—the term "microbiome" refers to the catalog of the microbiota's genes.[2] However, other authors have suggested that "microbiome" can mean "the entire habitat, including the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses), their genomes, and the surrounding environmental conditions."[3] This definition is based on the concept of a "biome"—the flora and fauna of a given environment—and that is the definition we shall use herein.

The microbiome is a complex system that differs from person to person like a unique fingerprint, but also between body sites and throughout the lifespan. It is estimated that there are hundreds of bacterial species on the skin including the most common: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*.[4] There is still much to be explored between the unique interplay of microbial colonization and disease development.

DEVELOPMENT OF MICROBIOME

Recent research suggests colonization of newborns may occur even before birth via hematogenous spread of microbes from the mother's oral cavity and gastrointestinal tract (e.g. *Firmicutes*, *Proteobacteria*, *Enterococcus*, *Streptococcus*) into the intrauterine cavity.[5] The birthing route also has major implications on the newborn microbiome and future disease risk.[5] It is well-established that children born by cesarean section may have altered skin and gastrointestinal flora.[5–7] Studies have demonstrated increased cutaneous colonization with *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.,[8] which may contribute to development of atopy.[9] Cesarean birth is often coupled with peripartum antibiotic use which decreases the presence of immunoprotective *Lactobacillus* and *Bifidobacterium* species in breastmilk.[5] Interestingly, infants who have greater presence of coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis, S. hominis*) have decreased development of atopic dermatitis (AD) and/or improvement in local eczema area and severity index scores.[10,11] These commensals are highly synergistic with the antimicrobial peptide LL-37 and together suppress *S. aureus* growth.[10,11] Another study assessing skin samples from the antecubital fossa of pediatric patients found an association between worsened disease severity and decreased bacterial community diversity (N=12, $p < 3.6e\text{-}04$).[12] Recent research has elucidated the

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role of specific commensals in improving microbiota composition. *Roseomonas mucosa* is a commensal gram-negative in the *Alphaproteobacteria* class and produces lipids that promote tissue regeneration and a shift away from *Staphyloccaceae*.[13]

Gut integrity and diversity also plays an important role in the development of inflammatory skin diseases. A study assessing fecal samples of 98 infants found that microbial diversity was significantly lower in infants with eczema compared to eczema-free infants at 12 months of age.[14] Specifically, the gut flora of children with AD contained more *Clostridia* and fewer *Bifidobacteria* and *Lactobacilli* species.[15] Both *Bifidobacteria* and *Lactobacilli* induce T regulatory cells and subsequent IL-10 and TGF-β production to attenuate inflammation and inhibit growth of *S. aureus*.[15–18] In addition to the well-known impaired skin barrier in AD, there may also be considerable gut barrier impairment. GI symptoms such as diarrhea and vomiting may also be associated with AD in children.[19] A randomized trial in pediatric patients aged 1-13 years found an association between severity of AD and increased lactulose-mannitol excretion ratio in urine samples, an indication of gut permeability.[20] Remarkably, after 6-weeks of treatment with an oral lactobacillus supplement, there was a significant decrease in the lactulose-mannitol ratio compared to placebo, signifying improvement in GI permeability and distressing GI symptoms ($p = 0.001$).[20]

PATHWAYS OF SKIN DYSBIOSIS

The skin is an important protective barrier, but disruption in a multitude of pathways can lead to disease. Individuals with AD have increased levels of inflammatory cytokines IL-4 and IL-13 inducing differentiation of Th2 (T-helper) cells and production of the immunoglobulin IgE.[21] This poses a problem in atopic individuals who have barrier dysfunction, allowing for entrance of cutaneous antigens and an allergenic response.[21] This creates a cycle for patients where allergen penetration promotes inflammatory mediators and IgE production, worsening the itch response and further damaging the skin barrier. This state leads to dysbiosis with increased colonization of *Staphylococcus aureus (SA)*. A number of studies have characterized the correlative relationship between *SA* burden and more clinically severe AD.[10,18,22–29]

SA contributes to dysbiosis through numerous independent pathways. The bacteria produces clumping factors A and B as well as fibronectin-binding protein which all promote pathogenic adhesion.[13] The bacteria also produce proteases which degrade the stratum corneum exposing the delicate dermal junction to potential immunogenic allergens.[13] SA also produces α toxin which leads to barrier destruction by forming pores in keratinocytes and allowing for biofilm production.[13] Given that AD patients already have a dysfunctional innate immune response (e.g. decreased antimicrobial peptides) on affected skin, biofilms makes it even more difficult to clear areas of adhesion.[13,30] As *SA* persists, factors such as Protein A are released from its cell wall surface triggering increased production of inflammatory cytokines.[13] The production of δ toxin further perpetuates the allergenic response by activating mast cells. Prevalence of *SA* carriage is >70% on lesional skin compared to 39% on nonlesional skin in AD patients.[13] This increased colonization correlates with disease severity including pruritus, sleep loss, and greater topical steroid use.[13]

Other factors besides *SA* virulence contribute to barrier stress. A shift in the pH to a more alkaline level promotes both barrier permeability and *SA* colonization.[13,31] In AD patients, studies have also demonstrated decreased levels of filaggrin, an endogenous moisturizing factor, and sphingolipids (e.g. ceramides, phospholipids, arachidonic acid).[6] This leads to drier skin and greater *SA* adhesion.[13,31] Overall, the pathway of skin dysbiosis is multifactorial and treatments that seek to improve barrier function and decrease the burden of *Staphylococcus aureus* will likely improve clinical outcomes for patients.

PATHWAYS OF GASTROINTESTINAL DYSBIOSIS

Recently, more attention has shifted toward the impact of the gastrointestinal microbiome both for digestive and inflammatory bowel diseases, but also its impact on the surface of the skin in atopic patients. General consensus agrees that a high number of species and genetic diversity is important for a healthy GI microbiome.[32,33] But the strains present are also critical: pro-inflammatory gut microbiota include a predominance of microbes from the Enterobacteriaceae family, including *Escherichia*, *Shigella*, *Proteus*, and *Klebsiella* likely due to the production of lipopolysaccharides (endotoxin).[32]

Several stressors including psychological stress, circadian disruption, diet composition, and antibiotic use affect the gut microbiota. During the biological stress response, catecholamines are released, which may modulate microbial growth.[32] Biological stress also increases the sympathetic response, moving blood away from the GI tract and resulting in hypoperfusion and oxidative stress that can impair the gut barrier.[32] While relatively limited, current findings demonstrate that psychological stress may be associated with microbiota changes including fewer *Lactobacillus* and reduced diversity.[32] Disrupting the circadian rhythm either through sleep deprivation or varying sleep schedules impairs rhythmicity of the gut microbiome and subsequent metabolic activity.[32] Sleep deprivation may alter the *Firmicutes:Bacteroidetes* ratio with an increase in *Firmicutes* increasing fat absorption and metabolism from the diet and a propensity to gain weight.[32] Interestingly, circadian disruption coupled with a highly processed (high-fat, high-sugar) diet may promote a greater increase in *Ruminococcus*, a genus associated with mucin degradation and gastrointestinal epithelial damage.[32] Antibiotics also have a deleterious effect on the gut microbiota including loss of diversity and important taxa, shifts in metabolic capacity, transfer of antibiotic resistance genes and increased colonization of invading pathogens.[34] A single course of antibiotics in infancy may alter the gut composition and diversity for several months.[35] After just one day of oral antibiotics, fecal samples displayed 50% decrease in *bifidobacteria* and replacement by *enterobacteria*, regardless of probiotic use. This depletion in *bifidobacteria* continued for at least 6 months and abundance of *Firmicutes* (within the *Clostridia*

class) remained 2.5-fold higher from before treatment. This replacement of bifidobacteria by high clostridial abundance has been linked with increased development of allergic diseases like eczema.[35,36] Decreased *bifidobacteria* also allows for colonization by *E. coli* which has been associated with an increased serum IgE level and risk of developing eczema.[33,36] Short-chain fatty acids including butyrate, propionate and acetate are produced by gram-positive anaerobes through conversion of high fiber plant foods in the diet. Short-chain fatty acids support the integrity of the intestinal barrier by upregulating tight junction proteins and attenuating pro-inflammatory pathways.[34,36,37] One study found milder atopic symptoms in patients with increased levels of short-chain fatty acids ($r = -0.59$, $p = 0.02$), signifying that improved gut integrity through dietary measures and avoidance of oral antibiotics may be important for this patient population .[36,37]

POTENTIAL TREATMENTS: RE-ESTABLISHING COLONIZATION

The effect of oral probiotic formulations on AD pathology has been assessed and the evidence is mixed. Depending on the strain, probiotics may stimulate anti-inflammatory cytokines like IL-10 and TGF-β and induce immune activation signaling through production of IL-12, IL-18 and TNF-α.[36]

Certain beneficial microbes such as *Lactobacillus* species and *Bifidobacterium* species produce γ-aminobutyric acid (GABA), which inhibits itch.[36] Supplementation with $1x10^{10}$ CFU of *Lactobacillus paracasei*, for example, has been shown to reduce skin sensitivity and transepidermal water loss in healthy adults.[38] One randomized trial found an initial reduction in eczema in infants aged 4-13 months given *Lactobacillus paracasei* 10^8 CFU versus placebo. However, follow-up at 8-9 years of age showed no difference in the number of AD patients in both groups suggesting only delayed onset.[39]

Lactobacillus rhamnosus GG is another lactic-acid producing bacterium and one of the most widely studied strains in the AD infant population. A systematic review of various nutrient supplements found moderate evidence for the use of *Lactobacillus rhamnosus GG* in mothers and infants for preventing development and reducing severity of AD.[40] However, Huang et al. conducted a systematic review of studies utilizing *Lactobacillus rhamnosus GG*, *Lactobacillus fermentum*, *Lactobacillus paracasei*, *Lactobacillus silvarium*, or a probiotic mixture of the strains. Pooled analysis of two studies administering *Lactobacillus rhamnosus* GG did not find a difference in outcomes ($p = 0.07$).[41] However, analysis of probiotic mixtures demonstrated a significant decrease in AD severity (pooled $p = 0.0009$).[41] Regarding single strain effects, Wu et al. demonstrated decreased SCORAD values in children aged 2-14 years treated with *Lactobacillus salivarius* ($2x10^9$ CFU) with fructo-oligosaccharide for 8 weeks ($p = 0.022$).[42] Similarly, one double-blinded randomized controlled trial provided either *Lactobacillus paracasei* ($2x10^9$ CFU), *Lactobacillus fermentum* ($2x10^9$ CFU) or the two strains together ($4x10^9$ CFU combined) to children aged 1-18 with moderate-to-severe AD for 4 weeks. The researchers found a significant reduction in SCORAD even after 3 months of probiotic discontinuation ($p < 0.001$).[43] A Cochrane review in 2008 found that probiotics slightly reduced investigator-rated eczema severity scores.[44] However, there was no evidence that probiotics impacted quality of life or patient- or parent-rated symptoms of AD.[44] Overall, many of these studies tested various strains and doses on a heterogenous patient population at variable lengths of time. This may dilute more convincing effects and suggest that there are variables that are not being considered. Randomized-controlled trials with set dosing, strain or strains, and careful patient selection may be necessary until we have a more precision-level understanding of the factors involved.

Topical probiotic formulations have also been studied for use in the AD patient population. One such study looked at the effect of topical application of *Roseomonas mucosa* from healthy volunteers in 10 adults and 5 pediatric patient AD patients (with an escalating dose of 10^3 to 10^4 to 10^5 CFU). While a small sample size, the study did show a significant decrease in SCORAD ($p < 0.01$, $p < 0.05$), regional pruritus ($p < 0.01$), and steroid application in both cohorts ($p < 0.05$). Further, there was a significant decrease in the relative proportion of SA in the antecubital fossa area of the pediatric cohort ($p < 0.05$).[45] Another open-label study utilized heat-treated *Lactobacillus johnsonii* NCC 533 lotion (0.93×10^9 CFU/mL) on 31 AD patients, 15 of which were SA carriers.[46] The use of the lotion twice daily for 3 weeks led to a statistically significant decrease in mean SCORAD for all participants and a statistically significant decrease of SA burden on treated lesions ($p = 0.012$, $p < 0.05$).[46] Another unique study utilized *Lactobacillus sakei* probio 65 from Korean kimchi, which has been shown to decrease IL-4 and IgE levels.[47] In this double-blind, randomized split-body trial, 28 AD patients applied the *Lactobacillus*-containing emollient and a control emollient on each side of their body twice daily for 4 weeks.[47] The treated sides had significantly lower trans-epidermal water loss and visual analog score of pruritus compared to the control sides, indicating improved barrier function ($p = 0.007$, $p = 0.006$).[47] Similarly, a double-blind, placebo-controlled trial evaluated the effects of *Vitreoscilla filiformis*, a gram-negative bacterium found in thermal spring water (5% bacterial lysate in control cream).[48] Among the 75 AD patients treated, there was a significant improvement in SCORAD ($p = 0.0044$), pruritus ($p = 0.0171$), and sleep loss ($p = 0.007$). On the other hand, one study split 34 AD patients and assessed the addition of *Lactobacillus reuteri* DSM 17938 in a novel barrier-protecting ointment ($1x10^8$ CFU/gram) compared to ointment alone.[49] Both groups applied the respective ointment twice daily for 8 weeks and had a clinically significant decrease in SCORAD compared to baseline ($p < 0.001$), however there was not a significant difference between groups.[49] Both products also reduced pruritus (-58% for probiotic and -34% for control) and sleep loss (-78% for probiotic and -76% for control). This indicates that the novel ointment alone may have accounted for the clinical improvement. Overall, the evidence for topical probiotics, whether live or heat inactivated, is promising and atopic patients may have clinical improvement with application. It is extremely important to differentiate between viable bacteria (probiotics in

the strictest sense) and non-viable heat inactivated bacteria, sometimes referred to as "Tyndallized", which are probably best referred to as "parabiotics". However, just as in oral probiotic supplementation, larger-scale, blinded randomized controlled trials are needed.

Because there are so many complexities, it follows that re-establishing a robust microbiome may not be as simple as a single probiotic strain or probiotic formulation. One small study assessed the effect of various topical agents on microbial colonization in vitro before testing the most efficacious compounds on a small group of healthy patients (N=3).[50] A combination of colophonium (pine tar), fusidic acid (antibiotic), and butyl paraben increased levels of *Alphaproteobacteria* (class containing *R. mucosa*) in vivo. The influence of this combination on *S. aureus* could not be assessed since the patients were not colonized, although it did not impact growth of coagulase-negative *Staphylococcus* species. Fragrance mix II, a combination of α-hexylcinnamicaldehyde, citral (lemon myrtle oil), citronellol, coumarin, farnesol and lyral also enriched levels of *Alphaproteobacteria* and also potentiated growth of coagulase-negative *Staphylococcus* spp. Interestingly, the combination of colophonium, fusidic acid and butyl paraben inhibited the fungal class *Malasseziomycetes* which is likely involved in AD pathology.[51,52] Colophonium alone and Fragrance Mix II did not impact the abundance of this fungal class. Overall, the results of this study indicate that combinations of various compounds may provide a synergistic effect on microbial diversity, although larger studies are needed with assessment of atopic patients. Many of these compounds are present in various cosmetic products and medicinal preparations that are used daily on the skin, underscoring the manifold factors that influence the cutaneous microbiome.

FUTURE DIRECTIONS

The evidence for both oral and topical probiotic formulations is increasing and may prove a viable treatment option for eczema patients. However, future studies must consider trialing multiple interventions at once as the microbiome is tremendously complex. Eczema disease severity is impacted by both internal gastrointestinal colonization and external environmental factors like SA load and presence of commensals. For example, moisturizer alone was shown to reduce AD severity by decreasing *Staphylococcus* genus and increasing *Xanthomonas* genus (Proteobacteria phylum).[53] Similarly, application of coconut oil decreases SA colonization and subsequent SCORAD in AD patients likely due to broad-spectrum antimicrobial activity.[54,55] Other technologies such as specific antimicrobial enzymes have demonstrated bactericidal effects against *SA* by disrupting cell wall integrity.[56–58] Research among these newer agents may be harnessed to manipulate the microbiome in more targeted ways.

Beyond microbial colonization, both gastrointestinal and skin barrier integrity remain vital foundational considerations. Research on the use of dilute bleach baths demonstrates improved trans-epidermal water loss and reduction in pruritus but does not significantly alter the skin microbiome.[26,28,59–63] Future research may aim to tackle disease severity through these various mechanisms by accounting for nutritional factors, moisturizer utilization, oral and topical probiotics as well as commensal application, among others.

Moreover, future treatment protocols may center around creating a "fingerprint" or personalized microbiome analysis for each patient. When a patient presents with a serious infection, antibiotics are selected based on testing for drug susceptibility to the pathogen. Similarly, having knowledge of an individual's microbiome makeup and specific deficits through skin and fecal sampling may better inform practitioners of optimal therapeutic interventions. Overall, there is tremendous potential to improve the microbiome, both on the skin and in the gastrointestinal tract among the AD population. It is exciting to continue to follow the advancements that are made and new protocols that will tackle the pathogenesis of eczema through multiple mechanisms.

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