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Skin microbiome of atopic dermatitis

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ABSTRACT

The skin microbiome is a key component of pathogenesis in atopic dermatitis (AD). The skin of AD patients is characterized by microbial dysbiosis, with a reduction of microbial diversity and over-representation of pathogenic *Staphylococcus aureus* (*S. aureus*). Recent exciting studies have elucidated an importance of establishing an appropriate immune response to microbes in early life and uncovered the new mechanisms of microbial community dynamics in modulating our skin microbiome. Several microbes are associated with AD pathogenesis, with proposed pathogenic effects from *S. aureus* and *Malassezia*. The complex relationships between microbes within the skin microbiome consortia includes various species, such as *Staphylococcal, Roseomonas* and *Cutibacterium* strains, that can inhibit *S. aureus* and are potential probiotics for AD skin. Numerous microbes are now also reported to modulate host response via communication with keratinocytes, specialized immune cells and adipocytes to improve skin health and barrier function. This increased understanding of skin microbiota bioactives has led to new biotherapeutic approaches that target the skin surface microenvironment for AD treatment.

Introduction

The skin is an important interface that constantly interacts with, senses our environment and has evolved to maintain a homeostatic relationship with a community of microbes that form the microbiome. Atopic dermatitis (AD) is a pruritic inflammatory skin barrier disorder that affects approximately 20% of children, and 10% of adults in developed countries.[1] AD is characterized by a defective barrier, which exacerbates microbiome stimulation of the immune system, and the combination of these factors plays major roles in AD pathogenesis. The complexity of pathophysiological drivers in AD underlies the heterogeneity of clinical presentation, such as age of onset, disease severity, natural course of disease and the flare cycle, which has distinct microbiome alterations and overgrowth of *Staphylococcus aureus (S. aureus)*.

Microbes coexist with humans and play an important role in regulating health and disease. In the last decade, advances in microbial sequencing technologies have provided insight on the multi-kingdom skin microbiome composition and characteristics across the spectrum of skin health.[2,3] Moreover, the skin surface has a wide range of microenvironments with unique physicochemical properties, selecting for distinct groups of microorganisms adapted to the niche they occupy across body sites.[3,4] This results in sebum-rich areas having an enrichment of *Cutibacterium* spp. and fungal *Malassezia* spp. which are reliant on lipids, and moist skin sites colonized by more *Staphylococcal* and *Corynebacterium* species due to greater nutrient availability.[4–9] The collective microbial composition constitutes our skin microbiota. Most of these microbial community members are commensals with growing evidence for symbionts that confer host benefits ranging from protection against pathobiont invasion, and educating of host immune response to respond appropriately against pathogenic microbes that may break through the skin barrier.[3] Microbiome studies in AD patients have consistently shown that lesional skin sites are abundantly colonized by *S. aureus* and have low microbial diversity. Therefore, mechanistic understanding of how microbial communities contribute to AD across the flare cycle can offer patients an additional therapeutic avenue to maintain skin health.

Additionally, there are strong underlying genetic contributions as shown by numerous GWAS studies which flagged up over 30 risk loci for AD, with filaggrin null mutations and T helper 2 (Th2) signalling molecules being significantly associated with AD.[10–12] A hallmark of AD is its strong association with an increased activation of the Th2 immune response, which includes increased infiltration of CD4+ cells and elevated levels of prototypical Th2 cytokines. Furthermore, Th2 overactivation can impair the function of important skin barrier proteins such as filaggrin and

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loricrin, exacerbating an already impaired skin barrier.[13,14] Although AD is predominantly Th2-skewed, there is heterogeneity in the immune dysregulation profile of AD patients, with some patients presenting an increased Th17 polarization as well as Th2 activation.[15–18]

Microbes and atopic dermatitis

S. aureus colonization aggravates skin barrier dysfunction

The skin microbiome in healthy individuals is generally stable over time,[2] but patients with AD display strong dysbiosis especially during flares, characterized by a reduction in microbial diversity.[19,20] Amongst the Staphylococcal species, *S. aureus* is often found enriched in AD, with the extent of *S. aureus* colonization correlating with the severity of AD.[19,21–24] *S. aureus* isolates from AD patients also express higher levels of virulence factors,[23,25] and have a greater propensity for producing biofilms to help promote its colonization[26–28] and immune evasion.[26] *S. aureus* is a gram-positive opportunistic bacterium that causes both superficial and invasive skin infections. It expresses various factors promoting colonization and virulence, including superantigens (SAgs) like enterotoxins (i.e. SEA, SEB), toxic shock syndrome toxin 1 (TSST1), exotoxins, phenol soluble modulins (PSMs), as well as proteases that trigger inflammation alongside skin barrier dysfunction in AD as reviewed[29–32] (Fig. 1).

An altered skin barrier is susceptible to *S. aureus* colonization,[33,34] as there is a preferential binding of *S. aureus* to AD skin due to a redistribution of fibronectin in the stratum corneum of AD skin.[35] AD patients with filaggrin (*FLG*) mutations typically harbor less FLG-breakdown products such as urocanic acid and pyrrolidone carboxylic acid, leading to an increase in pH favoring proliferation of *S. aureus*. Increased pH also promotes the expression of secreted and cell wall-associated proteins involved in immune evasion and adherence (i.e. clumping factor B, fibronectin-binding protein), further aiding the colonization of *S. aureus* on AD skin.[36] Upon colonization, *S. aureus* virulence factors driving AD pathogenesis causes further breakdown of the skin barrier and immune stimulation.

Cutaneous malassezia can drive AD pathogenesis

Malassezia spp. are the most abundant fungi on mammalian skin and are associated with AD.[37,38] *Malassezia* spp. exists as a commensal on healthy skin but overgrowth of multiple *Malassezia* spp. such as *Malassezia furfur* and *Malassezia sympodialis* among

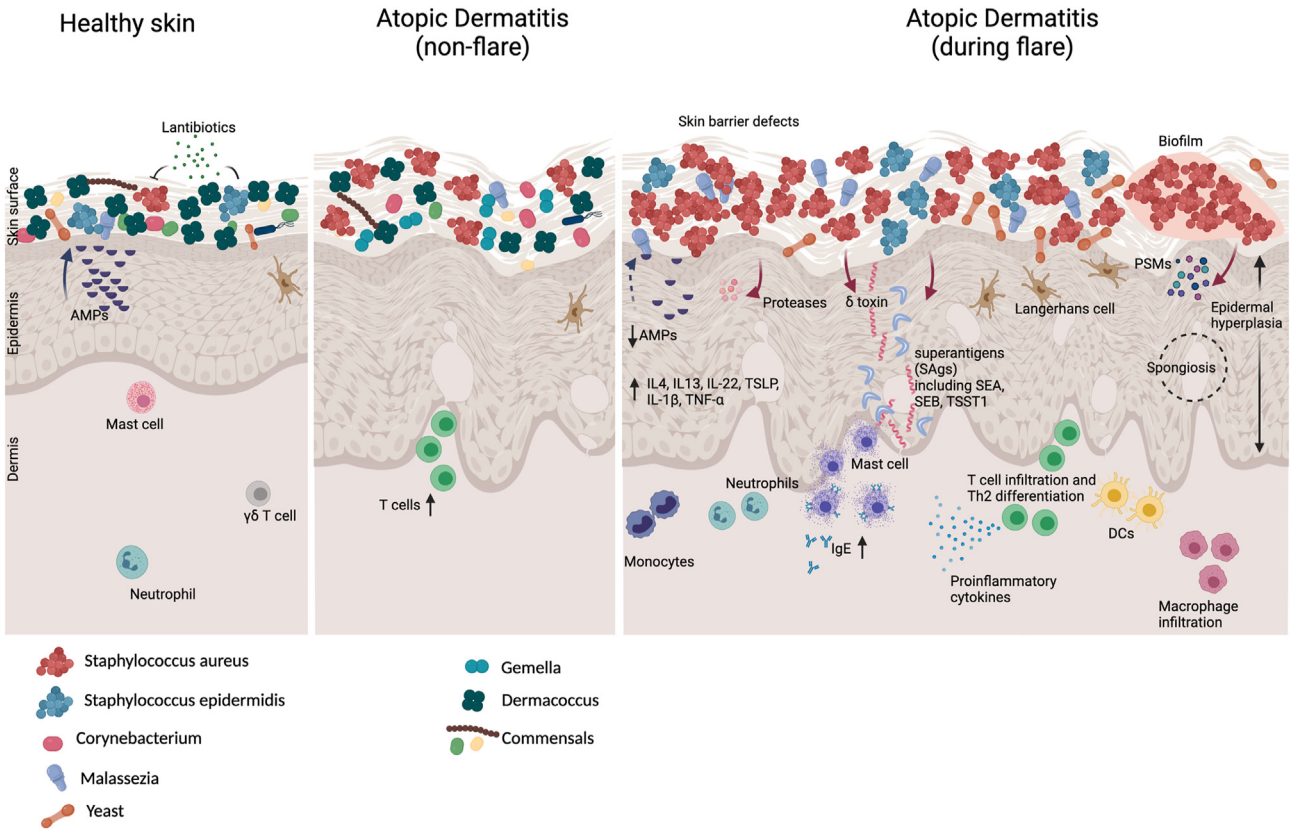

Fig. 1. Crosstalk between skin and microbiome in healthy and atopic dermatitis conditions. The presence of commensals on the skin interacts with the host to establish a functional immune response and prevents the overgrowth of pathogenic microbes. On healthy skin (Left), there is high microbial diversity, which includes *Dermacoccus* and *Corynebacterium* as well as other commensals. Secretion of AMPs and production of lantibiotics by commensals shape the microbial community on healthy skin to prevent colonization of pathogens like *S. aureus*. On AD skin in a non-flare state (Middle), microbial dysbiosis occurs with a reduction of *Dermacoccus* and an increased abundance of *Streptococcus* and *Gemella* species. The skin of AD patients during flares (Right) is characterized by the overgrowth of pathogenic microbes such as *S. aureus*, and reduced microbial diversity. Biofilm production by *S. aureus* promotes its colonization and drives pathogenesis. Commensals including *Dermacoccus* are depleted. Epicutaneous defects can be exacerbated by genetic mutations of skin barrier proteins such as filaggrin, *S. aureus* virulence factors, and Th2 cytokines. *S. aureus* virulence factors including δ-toxin and superantigens trigger IgE-mediated mast cell degranulation, and the resulting increase in Th2 cytokines perpetuates AD. Concurrently, there is a marked reduction of AMPs expressed, leading to the overgrowth of pathogens such as *S. aureus* and *Malassezia*.

others, may cause AD pathogenesis.[6,39–42] There is increased levels of *Malassezia*-specific IgE in AD patients[43] and a correlation of AD severity with amount of *Malassezia* species present.[44] Several studies have elucidated pathogenic mechanisms driven by *Malassezia*. *M. sympodialis* releases extracellular vesicles that can induce IL-4 and TNF-α cytokines in AD patients.[45] *M. sympodialis* can also induce the release of cysteinyl leukotrienes in IgE-sensitized bone marrow-derived mast cells (BMMCs) and enhance IgE-mediated degranulation of BMMCs. As the skin barrier is often defective in AD patients, percutaneous sensitization with allergens produced by *M. sympodialis* then perpetuate inflammation through mast cells activation.[46] Fungal protein MGL_1304, secreted by *Malassezia globosa* and found in the sweat of AD patients, causes type 1 allergy in AD patients.[47] And IL-23/IL-17 pathways are found to be important for maintaining a healthy balance for *Malassezia*-host coexistence; IL-23/IL-17 pathways exert protective functions that prevent the overgrowth of skin fungus colonization but these exact pathways can also exacerbate *Malassezia*-induced inflammation in atopic conditions.[48]

Community dynamics and skin commensals

Temporal stability in skin microbiome of healthy individuals suggests strong selective control on the microbial constituents. This can be driven by host factors (e.g. skin physiology), environmental exposure (e.g. UV exposure, temperature), and products secreted by other microbes through interactions or metabolism.

Within the Staphylococcal species, the coagulase-negative Staphylococci (CoNS) commensals such as *Staphylococcus epidermidis*, *Staphylococcus hominis*, and *Staphylococcus lugdunensis* are of importance. Certain strains of *S. epidermidis* were found to carry and express the gene that produces serine protease glutamyl endopeptidase (Esp), which inhibits the production of biofilms and colonization by *S. aureus*.[49] This inhibitory effect of Esp turns bactericidal, when Esp acts together with beta-defensin 2 (hBD2),[49] a host antimicrobial peptide (AMP). Interestingly, strains capable of attenuating *S. aureus* were typically found in subjects with low to no *S. aureus* carriage, hinting at a competitive exclusion between commensals and pathobionts. *S. lugdunensis* was found to efficiently inhibit *S. aureus* growth through the production of lugudinin, a novel class of macrocyclic thiazolidine peptide antibiotics.[50] A follow up study has demonstrated lugudinin's ability to promote immune responses through inducing host production of AMP cathelicidin (LL-37) and enhance the recruitment of phagocytes. In addition, lugudinin was shown to act synergistically with LL-37 and dermicidin-derived peptides, potentiating lugudinin's antimicrobial effects against *S. aureus*.[51] Following a screen of CoNS isolates collected from the skin of healthy and AD subjects, *S. epidermidis* and *S. hominis* were found to possess antimicrobial activity against *S. aureus*. Selected strains from both species were able to produce lantibiotics efficacious against *S. aureus*, but did not affect the growth of other commensals, highlighting its therapeutic potential in treating AD. *S. hominis* strain ShA9 was also shown to be capable of effecting host protection in an AD mouse model through lantibiotic-independent means by induction of AMP LL-37 gene expression. Concomitantly, Th2 and Th17 T cell recruitment was suppressed; highlighting ShA9's potential in regulating host immune responses.[52]

Ito *et al.* used a mouse model of dermatitis, with a variety of agents to induce skin inflammation including *S. aureus*; MC903, a vitamin D3 analog that models AD; and Imiquimod, a TLR7 agonist that models psoriasis-like dermatitis. In these models, *Staphylococcus cohnii* treatment showed amelioration of dermatitis and skin inflammation, along with suppression of immune responses including upregulation of anti-inflammatory glucocorticoid genes, and induction of immunoregulatory molecules. Furthermore, different isolates with slight alterations in microbial genome harbored differential immunomodulatory capacities, showing the potential of *S. cohnii* strains as a biotherapeutic.[53]

In addition to CoNS, other non-staphylococci microbes have shown antagonistic properties towards *S. aureus*. For example, *Streptococcus* spp. was shown to have an inhibitory effect on *S. aureus* growth,[20] while *Corynebacterium* spp. was shown to constrain *S. aureus* through accessory gene regulatory (agr) quorum sensing inhibition.[54] *Malassezia* can act as a protective commensal. Metagenomic profiling of AD patients show that *Malassezia* is depleted from the skin of AD-susceptible individuals.[20] Skin commensal *M. globosa* secretes protease MgSAP1 to restrict *S. aureus* biofilm formation by hydrolyzing *S. aureus* protein A, a virulence factor that is important in immune evasion and biofilm generation.[55] This shows a mechanism of a fungal–bacterium interaction, illustrating how various microbial community members can influence each other.

Not all commensals confer protection against *S. aureus*. While *Cutibacterium acnes* was shown to inhibit the growth of *S. aureus* with byproducts from fermentation of glycerol,[56] it was also found that some *Cutibacterium* species (including *C. acnes*) could facilitate *S. aureus* colonization by promoting *S. aureus* aggregation, and biofilm formation.[57] Chng *et al.* identified a microbial signature comprising of *Streptococcus* spp., *Gemella* spp., and a depletion of *Dermacoccus* spp. In subjects prone to AD. In the same study, it was found that the skin microbiome of these subjects has increased capacity for ammonia production,[20] suggesting that the metabolic activity of commensals may predispose individuals to *S. aureus* colonization.

The non-lesional skin and non-flare states of AD presents a state, which is in between that of a healthy skin and diseased lesional skin. Non-lesional skin is altered from normal skin as there is an increased presence of inflammatory T cells, decreased hydration, impaired synthesis of lipids, altered expression of differentiation markers. Non-lesional skin also has a similar transcriptional profile as lesional AD skin, although it expresses less immune-mediated inflammation compared to lesional skin[58] (Fig. 1). Our work on the skin microbiome of AD-prone individuals defined microbial signatures of AD susceptibility, that were termed “Dermotypes”, highlighting a distinct microbial community on the skin of AD patients during a non-flare state and altered skin surface components.[20,59,60] Interestingly, one of these dermotypes was associated with the AD endotype that has high IgE, so called “extrinsic AD”. This clinically more severe dermotype is characterized by reduced microbial diversity, depletion of *C. acnes*, *Dermacoccus* and *Methylobacterium* species, along with overgrowth of *Staphylococcus* species. It remains to be seen what is driving these various endotypes and dermotypes in AD patients that provides differences in allergic biomarkers and could be linked to the atopic march.

The complex host-microbe and microbe–microbe relationships that shape the eventual pathogenicity of each microbe is described in a concept introduced by Chen *et al.*, termed contextual pathogenesis. Suggesting that all microbes in the body fall somewhere within the spectrum of being potentially pathogenic (aggressive) and mutualistic (passive), with some microbes being generally beneficial to the host, but under certain circumstances can turn invasive, while some microbes may primarily be virulent.[61]

Skin microbiota of pediatric AD patients

As AD is a disease that has an early onset in the early years of life, studying the skin microbiome in this time scale is very relevant. Shi *et al.* investigated the skin microbiome differences between pediatric and adult AD patients. Their cohort includes young children aged

2–12, teenagers aged 13–17 and adults above 18 years old. The healthy and AD non-lesional skin microbiome of children was significantly more diverse compared to adults. AD lesions of children and adults were significantly decreased in microbial diversity, with greater abundance of *S. aureus*.[62] Similarly, Meylan *et al.* assessed the skin microbiome of infants and found those that developed AD had an increased *S. aureus* prevalence on their skin during the onset of AD at 3 months old, and 2 months before the onset of AD.[63] In a group of AD pediatric patients aged 2–18 years old, Byrd *et al.* found overgrowth *S. aureus* clonal strains abundance correlated to severe AD flares and heterogenous *S. epidermidis* strains correlated with less severe AD.[23] Nakamura *et al.* found that although the colonization with *S. aureus* on the cheeks of infants at 1 month old did not predict AD outcome, at 6 months old skin colonization by *S. aureus* could predict the risk of developing AD. 6 months old infants who did not develop AD had *S. aureus* strains with impaired agr function rendering them unable to grow and colonize.[64] In contrast, Kennedy *et al.* did not detect *S. aureus* in the AD lesions of their pediatric cohort, which was tracked at 4-time points; day 2, month 2, month 6, and 1 year old. They found that in the first year of life, infants who suffer from AD did not differ from control infants in terms of their Shannon diversity and neither had their skin colonized by *S. aureus*. The most significant difference between the AD and control infants were that infants affected with AD at 12 months old had significantly fewer commensal staphylococci compared to control infants, suggesting a potentially protective function of commensal staphylococci against AD development.[65] Taken together, the skin microbiota of pediatric AD patients may not always be characterized by the presence of *S. aureus* colonization or reduced microbial diversity, and this could be because microbiome is still developing throughout the first year of life.[66]

Microbes and immunity

Patients with primary immunodeficiency disorders commonly have eczema

Patients suffering from primary immunodeficiency (PID) diseases commonly have skin disorders, including AD.[67] Oh *et al.* examined the skin microbiome of PID patients who have a common feature of suffering from AD-like eczema and recurrent microbial infections, despite the different immunological deficiencies.[68] The PID patients examined include those with hyper-IgE (STAT3-deficient), Wiskott-Aldrich, and dedicator of cytokinesis 8 (DOCK8) syndromes. The skin of PID patients have increased permissivity to bacterial and fungal colonization, dysbiosis in microbial diversity, and unique bacterial-fungal colocalization compared to healthy controls. PID patients show a correlation of microbial presence and skin disease severity, and in addition to *S. aureus*, *Clostridia* spp. and *Corynebacterium* spp. also correlate with disease presentation. In contrast to AD patients who have significant overburden of *S. aureus*, PID patients primarily have *S. epidermidis* and *Staphylococcus haemolyticus*, shown capable of driving AD as well.[68] Skin microbial dysbiosis on PID patients indicate that crosstalk between our immune system and commensals impacts the microbial community on the skin.

Host immune responses to skin microbes

Neutrophils are a major part of the host innate immune response against pathogens, and *S. aureus* has been reported to inhibit effector functions of neutrophils, including prevention of recruitment by blocking extravasation, suppressing priming, activation, and phagocytosis, as well as neutrophil extracellular traps (NETs) formation.[69,70] The investment of resources by *S. aureus* to evade neutrophil effector functions suggests that this cell type is important in host protection. Indeed, neutrophils were demonstrated to be responsible in early control of *S. aureus* numbers on the superficial layers of the skin, thereby preventing *S. aureus* colonization.[71] Interestingly, in a separate study, *in vivo* NET formation by neutrophils was found to aid *S. aureus* colonization through an unidentified mechanism.[72] While both studies utilized epicutaneous model of *S. aureus* infection, Bitschar *et al.* involved tape stripping to induce skin inflammation rather than passive topical application by Schulz *et al.*, suggesting that the microenvironment (e.g. inflammation versus homeostasis) plays a role in affecting neutrophil's control of *S. aureus* colonization.

It has also been shown that γδ T cells secrete IL-17 and IL-22 that restrict *S. aureus* infections.[73,74] These cytokines control *S. aureus*-driven abscesses, influence T cell and neutrophil infiltration, as well as preventing *S. aureus* colonization.[75] Loss of conventional dendritic cells (cDCs) exacerbates AD skin inflammation and accelerates *S. aureus* colonization, thereby implicating cDCs in maintaining immune homeostasis, and preventing *S. aureus* colonization.[76]

In addition to immune cells, keratinocytes respond to *S. aureus* infection by increasing metabolic stress, with HIF1α activation. HIF1α signaling led to the production of inflammatory cytokines such as IL-1β and IL-18, which are associated with inflammasome activation. Inhibiting glycolysis resulted in decreased IL-1β production, and a larger *S. aureus*-induced skin lesion with delayed healing. Hence, the role of glycolysis in immunometabolism is important to control *S. aureus* overgrowth.[77] Human dermal adipocytes were also observed to regulate the invasion of *S. aureus* by producing LL-37. Inhibiting adipogenesis reduced LL-37 production, and increased susceptibility to *S. aureus* infection,[78] demonstrating the role of adipocytes in innate immunity against *S. aureus*.

Early exposure to commensals trains the immune system

Commensal microbiota have a protective function in early life, as reduced exposure to microbes increases susceptibility to AD and other allergic conditions in early childhood.[65] Neonatal skin colonization by commensal *S. epidermidis* induces immune tolerance through specific regulatory T cells (Tregs). In contrast, *S. aureus* minimally induces the development of specific Tregs and this is in part due to *S. aureus* α-toxin's activation of IL-1β.[79] A diverse community of skin commensals is therefore important to educate the immune system, and allow the host to develop tolerance and be able to appropriately respond to environmental stimuli.

Immune education begins *in utero*; the gestational environment is not sterile[80–85] and the presence of microbes in the gestational environment influences the fetal immune system. Mishra *et al.* detected live, culturable bacterial strains in fetal tissues that are capable of triggering activation of memory T cells *in vitro*, indicating that immune priming, and the interplay between microbiome and immune regulation may already commence during gestation. Studies from the same group demonstrated vertical transmission of allergic disease through maternal IgE sensitization of fetal mast cells.[86] These findings of *in utero* immune priming and maternal–fetal transmissions may explain in part, the observation that family history of atopy is the strongest predictor of AD risk in children,[87,88] as the contributions of both maternal immune[86] and microbial components[85] potentially shapes early immune responses to microbes.

Microbiome-based biotherapeutics

Targeted therapies to treat AD microbiota dysbiosis

Next generation microbiome-based biotherapies have been gaining traction over the past decade and are set to be a major

growth area for new treatments for AD. The classical approach to treating *S. aureus* infection in AD is through decolonizing using broad-spectrum antibiotics that comes with risks of promoting antibiotic resistance and importantly, perturbing the commensal microbes.[89,90] *S. aureus* can also use mechanisms to evade antibiotic killing.[91] Dilute bleach baths were trialed as a potential accessible method to manage *S. aureus* abundance, but results from multiple trials did not show a consistent effect on alleviating AD,[92] and the therapeutic effects that bleach baths have shown may not be due to its antimicrobial effects.[93] Hence, more targeted therapies such as microbiome-based options are needed that aim to restore a healthy skin microbiome in AD patients, reduce overgrowth of pathogenic drivers of AD and promote the recovery of commensals. These therapies include probiotics treatment, repopulating AD lesions with beneficial commensals, phage therapies, small molecules and peptides that counteract *S. aureus* colonization, humanized monoclonal antibodies that target bacterial toxins, as well as quorum sensing inhibitors that block virulence factors[94–96] (Fig. 2).

Probiotic and repopulating AD lesions with commensal microbes

Restoring a diverse microbiota that includes commensals will improve the community resistance to colonization with pathobionts. *S. epidermidis* application on healthy individuals increased skin lipid content and lowered skin acidic conditions.[97] As epidermal lipid composition and an acidic skin pH correlate with a healthy skin microbiome composition and diversity,[20,98] populating AD lesions with *S. epidermidis* can encourage optimal lipid levels and acidic skin pH to promote healthy skin microbiota. Furthermore, Bayer AG, Germany and Azitra Inc., USA partnered to develop skin care products with selected *S. epidermidis* strains for AD prone skin showing how topical application of commensals can be harnessed for the lucrative skin care market. Next, *S. epidermidis* and *S. hominis* strains with antimicrobial activity against *S. aureus* were isolated and treatment of AD patients could decrease *S. aureus* colonization.[99] *S. hominis* ShA9 can selectively kill *S. aureus* and promote beneficial bacteria to act as a bacteriotherapy for AD.[52] AD patients who received a 1-week treatment of topical ShA9 had fewer adverse events related to AD, significant decrease in *S. aureus* abundance, inhibition of PSMα expression by *S. aureus*, but did not reduce AD severity. Microbial strain differences between isolates derived from healthy versus AD patients are important to the outcome of microbial therapies. Treatments with isolates of culturable Gram-negative bacteria (CGN) obtained from healthy individuals, but not AD patients, correlated with an improved skin barrier and prevented *S. aureus* colonization.[100] A first-in-human trial used live topical *Roseomonas mucosa* from healthy individuals to treat adult and pediatric AD patients[101] to alleviate AD (reduced SCORAD, pruritis and use of steroids). The alleviation of AD symptoms was through the induction of a TNR-related epithelial pathway.[102] A potential therapy combining three strains of *R. mucosa* (FB-401) is currently being developed by Forte Bioscience.[96] *Nitrosomonas eutropha* (B244) is a bacterium that produces nitric oxide, a potential anti-inflammatory molecule. Results from

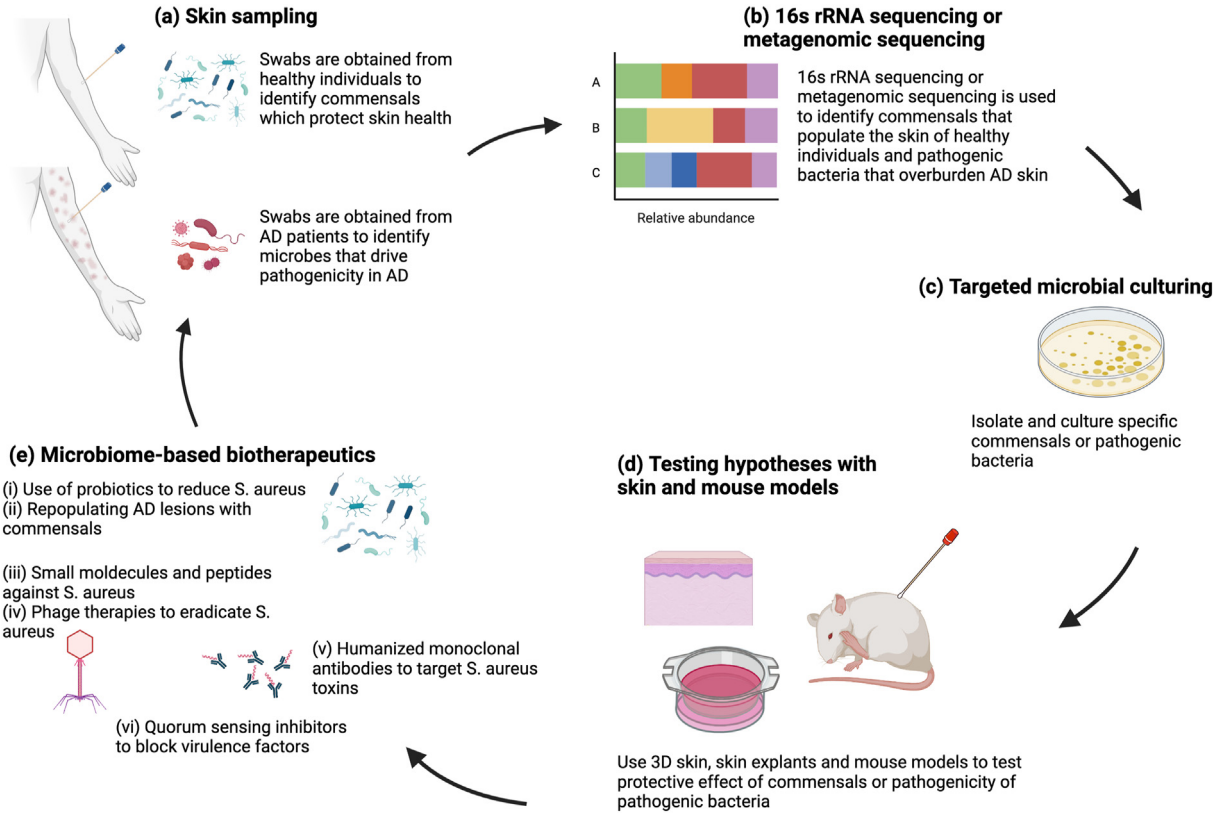

Fig. 2. The process of bench to bedside for microbiome-based biotherapeutics for AD. **(a)** Skin sampling: Skin biomass is obtained from AD afflicted sites (e.g. antecubital fossa) of healthy individuals and AD patients. **(b)** 16s rRNA sequencing or metagenomic sequencing: Sequencing is performed to uncover microbial taxonomy and strain diversity information between healthy individuals and AD patients to identify microbes of interest; such as identification of commensals with certain characteristics or possible pathogenic bacteria that exacerbates AD. **(c)** Targeted microbial culturing to specifically isolate and culture strains of potentially pathogenic bacteria or commensals for downstream testing. **(d)** Testing hypotheses with 3D skin, skin explants, and mouse models to ascertain the mechanistic role of strain of interest. **(e)** Microbiome-based biotherapeutics include probiotics treatment, repopulating AD lesions with commensals, phage therapies, small molecules and peptides that counteract *S. aureus* colonization, humanized monoclonal antibodies that target bacterial toxins, and quorum sensing inhibitors that block expression of virulence factors.

clinical trials showed an improvement in pruritis and this treatment is being developed by AOBiome.[96] Also, the treatment of *Lactobacillus johnsonii* NCC533 is a potentially beneficial treatment in atopic skin, as topical treatments on *in vitro* organoid skin model showed reduced *S. aureus* adhesion and increased the expression of AMPs.[103]

Phage therapy, small molecules and peptides

Staphefekt SA.100 is a recombinant phage endolysin that binds to the cells walls of *S. aureus* and cleaves the bonds in the peptidoglycan walls to result in cell death. Clinical trials with topical Staphefekt SA.100 produced conflicting results. It was effective in suppressing clinical symptoms of AD in a small study of 3 AD patients,[104] but in a larger trial of randomized patients, endolysin treatment against *S. aureus* was not efficacious in reducing *S. aureus* abundance nor did it reduce topical corticosteroid requirement.[105] Clinical parameters of AD improved when a clinical isolate of staphylococcal phage, SaGU1, obtained from the skin of AD patients, was tested in combination with commensal bacteria *S. epidermidis* on the back skin of a contact hypersensitivity mouse model.[106] Phage SaGU1 is a potential AD therapeutic agent as it can infect many *S. aureus* strains that have been isolated from AD patients but does not kill commensal bacteria, such as *S. epidermidis* strains.[107] In the small molecule space, Niclosamide ATx201 has been shown to decolonize *S. aureus*, improve the skin barrier and reduce inflammation markers.[96] Synthetic peptides can also be effective against biofilms formed by *S. aureus*[108] and Omniganan (CLS001) is currently being developed as a drug for AD.[96]

Future perspectives

Measuring skin microbial diversity to monitor, stratify and treat patients?

Several studies have concluded that microbial diversity and *S. aureus* abundance correlates with AD severity.[22,60] The use of traditional AD therapies such as UVB results in an increase in skin microbiota diversity on lesional skin.[109] Likewise, recent biologic treatment regimens such as Dupilumab, also result in an increase in skin microbiota diversity and decreased *S. aureus* abundance.[110,111] Hence, using skin microbial diversity or the abundance of *S. aureus* could be a viable, objective measurement for AD severity compared to only SCORAD, the semi-quantitative scoring method that is currently widespread. Tracking the skin microbiome could be a non-invasive molecular diagnostic indicator for optimal treatment regime and skin health.[112]

AD patients that are colonized by *S. aureus* present a more severe disease and epidermal impairments.[24] Our laboratory has recently identified two dermotypes (Dermotypes A and B) to stratify AD patients. Dermotype B is characterized by patients with a higher IgE, more severe itch, recurrent flares and disease presentation, with reduced skin microbiome diversity that favors Staphylococcal species and *S. aureus* colonization.[20,60] As AD is a heterogenous disease, stratification by dermotypes coupled with monitoring changes to skin microbiota could aid in monitoring AD progression or recovery, which will contribute to a more personalized AD treatment linked to spatio-temporal skin condition.

Possibility of personalized therapies?

Advances in microbiome engineering is opening new possibilities in the study and treatment of diseases with microbial dysbiosis. Phages can be used to deliver CRISPR/Cas9 payloads with guides to target strains of interest. These guides can target genes with high specificity, allowing genes that drive pathogenesis in pathogenic strains to be genetically removed. This technique of gene delivery can allow the integration of potentially useful genes to restore antibiotic sensitivity and introduce biofilm dispersal enzymes. For the gut microbiome, Eligo Biosciences, France is moving to clinical trials with the "CRISPR Nanobits" combining phage and CRISPR-Cas technologies to edit specific microbes with skin treatments an obvious potential next step. Like phages, genetic manipulation can be introduced via plasmids and transposable elements. Conjugation-mediated genetic engineering has a broader host range and can transfer more complex genetic material compared to phage-mediated engineering.[113]

Using a mixed community of skin microbes to boost therapeutic potential?

At present, microbiome therapies to repopulate AD lesions with commensals have focused on delivering a specific strain of bacteria. Using human skin equivalents, Loomis *et al.* found that treatment with a mixed community of microbes resulted in significant changes in transcriptomic and histological analyses more than treatment with single microbes. Mixed community treatment was able to increase filaggrin expression, reduce actively proliferating cells and increase the thickness of the epidermis more robustly than a single microorganism, reflecting how we could tap into community dynamics to enhance microbiome-based treatments for AD.[114]

Concluding remarks

To summarize, the skin microbiome is a complex and constantly evolving community that interacts across the skin barrier to communicate with the host. *S. aureus* overburden and reduced microbiome diversity has been shown by many studies to be a characteristic of skin during AD flare. Emerging research have pointed to early life being a pivotal period for the development of appropriate immune response to microbes and this could be a critical time to initiate microbiome interventions. Investigations on microbial community dynamics to differentiate healthy skin and skin during AD flares, will drive innovation on how to sustainably treat microbial dysbiosis of AD patients, which has led to several clinical trials of biotherapeutics to correct the AD microbiome dysbiosis. Results show that microbiome-based therapeutics have multiple viable treatment possibilities for AD but the specific strains of commensals that would produce lasting effects remains to be fully investigated.

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Conflict of interest

The authors have no conflict of interest to declare.

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