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Staphylococcal Biofilms in Atopic Dermatitis

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Abstract

Purpose of Review—Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder that is a major public health burden worldwide. AD lesions are often colonized by *Staphylococcus aureus* and *Staphylococcus epidermidis.* An important aspect of *Staphylococcus spp.* is their propensity to form biofilms, adhesive surface-attached colonies that become highly resistant to antibiotics and immune responses, and recent studies have found that clinical isolates colonizing AD skin are often biofilm-positive. Biofilm formation results in complex bacterial communities that have unique effects on keratinocytes and host immunity. This review will summarize recent studies exploring the role of staphyloccocal biofilms in atopic dermatitis and the implications for treatment.

Recent Findings—Recent studies suggest an important role for biofilms in the pathogenesis of numerous dermatologic diseases including AD. *S. aureus* biofilms have been found to colonize the eccrine ducts of AD skin, and these biofilms influence secretion of keratinocyte cytokines and trigger differentiation and apoptosis of keratinocytes. These activities may act to disrupt barrier function and promote disease pathogenesis as well as allergen sensitization.

Summary—Formation of biofilm is a successful strategy that protects the bacteria from environmental danger, antibiotics, and phagocytosis, enabling chronic persistence in the host. An increasing number of *S. aureus* skin isolates are resistant to conventional antibiotics, and staphylococcal biofilm communities are prevalent on the skin of individuals with AD.

Compliance with Ethical Standards

Conflict of Interest Dr. Herr reports the following disclosures: Advisory board member for Hoth Therapeutics, Inc.; Owns equity in Chelexa BioSciences, LLC; Co-inventor on patent EP23106821 licensed to Chelexa BioSciences, LLC; and Co-inventor on patent application US 20140308326 A1. Ms. Gonzalez, Dr. Biagini Myers, and Dr. Khurana Hershey declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Staphylococcal colonization of the skin impacts skin barrier function and plays multiple important roles in AD pathogenesis.

Keywords

Atopic dermatitis; Biofilm; Staphylococci; Microbiome; Barrier function; Epidermis

Introduction

Atopic dermatitis (AD) is a chronic skin condition characterized by eczematous lesions and intense itching [1]. AD presents in about 10% of children and 7% of adults in the United States [2]. Industrialized countries have a higher prevalence of AD, although an increasing number of cases are observed in developing countries [3]. AD presents most commonly in early childhood, with up to 60% of patients developing symptoms within the first year of life [3]. Lesions commonly present on the face in infancy and progress to other sites of the body, particularly skin surfaces that are subject to flexure [1]. In adulthood, these lesions often undergo lichenification [1]. AD affects many facets of daily life for patients, families, and caretakers as itching, scratching, and loss of sleep significantly impact quality of life.

While dry, itchy skin can cause significant discomfort, this disorder is also characterized by a compromised barrier in the skin and possibly other epithelial surfaces, facilitating IgE sensitization to environmental allergens. Penetration of these allergens may contribute to the eventual development of allergic rhinitis and asthma later in childhood in many AD patients, a process known as the atopic march [4]. There are many contributors to the pathogenesis of AD including genetic susceptibilities and dysbiosis of the skin microbiota [3, 5–9]. Genetic predisposition to the development of AD involves genes expressing proteins that contribute to skin barrier function. The most common example is filaggrin, a structural protein that is incorporated into the cornified envelope, a highly crosslinked mixture of structural proteins that surrounds a network of keratin filaments in the outer layer of the epidermis [10]. Mature filaggrin is proteolytically processed from the profilaggrin precursor, which also releases amino acid degradation products that play a vital role in retaining moisture in the skin. However, in addition to the genetic predisposition to AD, there is accumulating evidence demonstrating the central role that the skin microbiome plays in the pathogenesis of AD. Ninety percent of patients with AD are colonized with *S. aureus* while only 5–20% of healthy individuals are typically colonized [1, 11]. A major challenge with *S. aureus* is its propensity to form biofilm, which contributes to increasing severity in many diseases [12, 13]. Importantly, staphylococcal biofilms were found to be nearly ubiquitous in AD lesional skin [14••]. Those biofilms are complex microbial communities that provide an advantage to the bacteria in terms of evading the host immune response and resisting antibiotic action. The objective of this review is to summarize what is currently known about staphylococcal biofilms and how they impact skin keratinocytes and influence host immune responses. We will review the basics of biofilms and biofilm formation, how skin-colonizing organisms interact with each other in biofilms, how biofilms may trigger and exacerbate AD, and how these recent developments influence potential directions for clinical management.

Epidemiology of Atopic Dermatitis and the Hygiene Hypothesis

The most valuable AD prevalence and trend data were collected by the International Study of Asthma and Allergies on Childhood (ISAAC), the only global study to use uniformly validated methodology to allow comparisons of populations worldwide [15]. The prevalence of eczema differs between developing and industrialized nations [16], with rates in industrialized nations increasing to as much as 15–30% of children and 2–10% of adults [17]. The ISAAC data was initially collected during 1994–1995 and re-collected 5–10 years later in 56 countries [18]. These data revealed that 58% of participating centers reported an increase in eczema prevalence among older children (13–14 years) [18, 19] and 84% reported increased prevalence of eczema among younger children (6–7 years), with the highest increases seen in Western Europe, Canada, South America, Australasia, and the Far East [18]. While these substantial differences argue that environmental factors and genetic predisposition are key players for eczema development worldwide [18], this also raises the possibility that that skin microbial fluctuations modulate the gene-environment interactions at the skin surface [20].

Although there is a general agreement that microorganisms are potential components of many skin disorders, there is limited literature about how they relate to the genetic and environmental variation that also contributes to the disease [20]. The association of AD with factors that are linked to microbial exposure, such as daycare attendance, living on a farm environment, household pets, endotoxin exposure, and early antibiotic use supports the microbial component of AD [15]. The “revised” hygiene hypothesis theorizes that a decrease in early childhood exposures to infection, and by extension microbial exposure, increases the susceptibility to allergic disease [15], suggesting that diversity in the early microbiota might be important in allergy development and prevention [21]. Other changes in lifestyle in industrialized countries, such as increased skin washing, not only leads to removal of harmful pathogens but also removes antimicrobial peptides produced by the keratinocytes to protect the skin barrier [22]. This concept is timely because it is predicted that two-thirds of the human population will be living in urban areas by 2050, resulting in declining contact with the natural environment [23, 24].

Barrier Function in the Skin

The skin acts as a barrier to environmental insults or allergens and is also a key in preventing water loss from the tissue. The epidermis, the upper layer of the skin, consists primarily of keratinocytes that are regenerated regularly. The skin can be subdivided into several layers with functional specialization (Fig. 1): the outermost stratum corneum, the underlying stratum granulosum, and the lower stratum spinosum and stratum basale [10]. Throughout life, skin is continually being regenerated through the migration of keratinocytes from inner layers outward to the stratum corneum. As keratinocytes migrate upward from the stratum basale through the epidermis, they differentiate and proliferate, lose their organelles, undergo cornification, and slough from the skin [4, 25]. Specifically, the stratum basale contains proliferating, undifferentiated keratinocytes; these migrate up into the stratum spinosum, where they cease proliferating and begin to differentiate. Keratinocytes in the stratum granulosum still contain organelles and are characterized by the presence of granules

containing keratohyalin. Finally, the outermost stratum corneum is comprised of dead, flattened keratinocytes that are crosslinked together by corneodesmosomes to form a waxy, dense barrier, a process called cornification [10].

AD can be characterized by defects in the skin barrier that predominate in the stratum corneum. Filaggrin (encoded by the gene *FLG*) is a structural protein that is crucial to the formation of an intact normal skin barrier; it condenses keratin fibers from the cytoskeleton into tight clusters [25] and is crucial to maintaining the physical strength of the stratum corneum. Mature filaggrin is the result of proteolytic processing of the profilaggrin precursor, which also results in the release of peptides that are essential for skin homeostasis, e.g., natural moisturizing factor (NMF), and for maintenance of an acidic pH [26].

The *FLG* gene is the most widely studied in AD; loss-of-function mutations, truncations, and null mutations have been identified as contributors to atopy [25]. Patients with filaggrin mutations exhibit perturbed barrier function as a result of the loss of structural integrity in the cornified envelope, which normally functions to minimize water loss and to protect the lower layers of the skin from exposure to external antigens or environmental factors. Although *FLG* defects predispose individuals to atopic conditions, sensitization is also necessary as shown in mice exposed to external antigens [27–29]. Both lesional and normal-appearing skin of individuals with AD have been shown to have abnormal barrier function, as demonstrated by elevated transepidermal water loss (TEWL) compared to healthy controls. These findings support the idea that AD is a disease of barrier dysfunction [30].

The epidermis is rich in various proteases with multiple targets that are under strict regulation. These proteases act to degrade superfluous proteins, activate downstream pathways that impact terminal differentiation, and cleave precursors of structural proteins or other proteases into mature, processed versions [31]. Given the tight regulation of skin barrier function by the balance between critical proteases such as the kallikreins and protease inhibitors such as SPINK5, it is no surprise that changes in protease levels or activity in the skin contribute to defects in barrier function seen in AD [32–35]. Recently, filaggrin knockdown keratinocytes were shown to have increased endogenous cysteine protease activity, suggesting that the epidermal phenotype observed in *FLG* deficiency may be due in part to unleashed cysteine protease activity. Indeed, when these cysteine proteases were inhibited, keratin and tight junction proteins were significantly rescued [36]. In addition to endogenous control of protease levels, exogenous factors can also modulate protease secretion in the epidermis. For example, *S. aureus* induces increased secretion of kallikreins by keratinocytes [37]. These serine proteases can act to degrade filaggrin as well as desmoglein-1, an important component of desmosomes [32, 37]. Cleavage of desmoglein-1 is a normal aspect of desquamation, but dysregulation of this process can lead to impaired barrier function. Different strains of *S. aureus* and *S. epidermidis* show varying effects on protease activity of keratinocytes, suggesting that a detailed understanding of staphylococcal colonization at the strain level will be important for understanding the impact on keratinocytes in AD patients.

Skin Microbiome

A crucial factor impacting skin barrier function and both local and systemic immune responses in AD is the skin-specific microbiome. Microbes can influence human health through interactions at host epithelial surfaces, including skin and oral, respiratory, and urogenital mucosae. The interaction of microbiota with the skin surfaces is extensive: including microbial colonization of skin follicles, the total surface area involved is estimated to be 30 square meters [38]. The skin microbiome is less well characterized than the gut microbiome; however, there is increasing interest given recent studies demonstrating that skin commensals influence host immunity [39–41]. The distribution of microbial communities changes with gender, age, and fluctuations in immune status [41] and is also sensitive to changes in humidity or seasonal weather [42]. In contrast, birthing method and feeding method have little effect on the skin microbiome [43]. The composition of the skin microbiome varies widely between different skin sites, dependent in part on whether the skin site is dry, oily, or moist [40, 44••].

The skin microbiome can fluctuate in various states of disease. Dysbiosis is observed in AD, with loss of microbial diversity and over-abundance of certain microbial species. *S. aureus* prevalence greatly increases during AD flares and decreases after the lesion resolves [44••]. To assess the evolution of dysbiosis in the skin microbiome, a study was conducted to characterize the skin microbiome within the first 6 months of life. Colonization at the antecubital fossa with commensal staphylococcal species (e.g., *S. epidermidis* and *S. cohnii*) at 2 months of age was associated with decreased incidence of AD at 1 year [43]. Children who had developed AD at 1 year of age showed a decrease in skin commensals, suggesting that these species are protective. Notably, *S. aureus* was not observed in any samples collected from infants before the onset of AD symptoms [43]. More studies are needed to assess when *S. aureus* colonization most commonly occurs and the implications it carries for inflammatory responses.

Recent developments in sequencing technology allow for assessment of microbial communities, including hard-to-culture organisms. 16S rRNA sequencing has been used in many studies to assess the taxa present in a microbial community of the skin. Although 16S rRNA sequencing is relatively affordable, this method typically resolves taxa down to the genus or species level and does not provide strain-level resolution of the microbiome [45, 46••]. Recent studies have emphasized that specific strains of *S. aureus* or *S. epidermidis* can differ in the expression of critical virulence or protective factors (e.g., proteases or antimicrobial peptides) that may play important roles in pathogenesis of AD. Thus, in order to fully understand strain-specific effects on atopy, shotgun metagenomic sequencing is necessary, since it can resolve species- and strain-level variation. Both 16S rRNA and shotgun metagenomic sequencing were recently used to verify that the relative abundance of *S. aureus* rises to a striking degree during AD flares and decreases after treatment [44••, 46••]. *S. epidermidis* abundance was also observed to increase during AD flares, but to a lesser degree. Byrd et al. showed that *S. aureus* strains varied between individuals, however, each individual typically was colonized by a single strain of *S. aureus. S. epidermidis* populations were shown to be more heterogeneous and could vary at different skin sites from the same individual [46••]; such variation is likely due to differences in the skin

microenvironment [40, 47••]. mice were colonized with *S. aureus* strains isolated from healthy controls and *S. epidermidis* from AD patients, non-inflammatory responses were elicited. However, severe inflammation was noted when mice were colonized with *S. aureus* strains isolated from AD patients [46••]. The same *S. aureus* strains also induced an influx of $CD4^+$ T cells and increased secretion of IL-13, demonstrating the ability of specific strains to elicit different inflammatory responses.

S. aureus in AD

S. aureus is known to initiate and aggravate inflammation in AD lesions by secreting a number of factors that modulate host immunity or compromise barrier function in the skin. Staphylococcal alpha toxin, a cytolytic secreted factor, induces cell death in keratinocytes, which is further potentiated in the presence of Th2 cytokines [48]. Furthermore, decreased expression of filaggrin increases the susceptibility of keratinocytes to cytolysis by alpha toxin, due to concomitant decrease in sphingomyelinase levels [49]. In addition to the production of toxins, *S. aureus* secretes a large number of proteases that are important virulence factors. Among these, the V8 protease and exfoliative toxins A and B have each been demonstrated to cleave desmoglein-1, a critical structural protein within the corneodesmosomes that anchor differentiated keratinocytes to one another [50–52]. Such proteases therefore degrade the barrier function in the skin, increasing water loss and allowing greater exposure to external antigens.

Greater than 80% of *S. aureus* isolated from patients with AD also secrete superantigens, such as Staphylococcal enterotoxin B (SEB) and Toxic Shock Syndrome Toxin-1 (TSST-1). These toxins crosslink MHC-II and T cell receptors leading to the hyperactivation of T cells. These superantigens lead to significant inflammation in AD and contribute to atopy, as specific IgE against these molecules is often observed [6]. Toxin-producing *S. aureus* also induces corticosteroid resistance in peripheral blood mononuclear cells (PBMCs) in vitro, as PBMCs stimulated with superantigen were resistant to dexamethasone [53]. In patients with AD, *S. aureus* isolates from patients that demonstrated corticosteroid resistance exhibited a greater ability to produce superantigens than isolates from corticosteroid-responsive AD or the general population [54].

Staphylococcal Biofilms in AD Skin

Epithelial surfaces are constitutively colonized by bacteria, which commonly exist in the form of biofilm communities. For example, *S. epidermidis* forms biofilms between squamous epithelial cells in normal skin that vary in thickness depending on the type of skin site (e.g., dry vs. moist), and they colonize sebaceous glands and hair follicles [55]. Furthermore, positive Congo red staining of the epidermis of patients with AD revealed that *S. aureus* biofilms exist in the eccrine ducts [14••]. Congo red typically stains amyloid proteins, which in the skin are normally found in the dermis as macular amyloid, but the matrix of staphylococcal biofilms contains amyloid and thus stains with Congo red as well. Among *S. aureus* and *S. epidermidis* isolates from AD patients, 85% were strong biofilm producers. Interestingly, while staphylococci were found across the body regardless of lesion site, biofilms were only observed in AD lesions [14••]. While the characterization of *S.*

aureus biofilms in the skin is at an early stage, the implications of these findings are intriguing, given that biofilms are associated with refractory, recurrent infections that resist immune responses and antibiotic treatment.

Basics of Biofilm Formation

Biofilms are a growth adaptation to environmental stressors; the biofilm growth mode confers resistance to immune defenses and antibiotics [56]. Biofilms are surface-attached microbial communities typically surrounded by extracellular polymeric substances (EPS). EPS is a composite of extracellular DNA, exopolysaccharides, and proteins unique to bacterial biofilms [57]. Staphylococcal biofilm formation begins by adherence of bacteria to a primary surface followed by accumulation of cells via intracellular adhesion mechanisms, and finally the formation of a mature biofilm [58, 59] (Fig. 2). Staphylococcal biofilms can attach to a variety of surfaces, including abiotic material and human tissues, including the skin [60]. The ability of *S. epidermidis* in particular to adhere to abiotic surfaces and form biofilms is the reason it is the species responsible for the largest number of device-related infections [61]. Both *S. aureus* and *S. epidermidis* express a large number of MSCRAMM (microbial surface component recognizing adhesive matrix molecules) adhesion proteins that mediate adherence to host extracellular matrix proteins [62–70]. Several of these staphylococcal MSCRAMM-matrix interactions are relevant in AD. For example, the stratum corneum of AD skin has increased fibronectin relative to healthy control skin, and *S. aureus* fibronectin-binding protein (FnBP) A and B can interact with fibronectin in human skin [71]. Likewise, the *S. aureus* MSCRAMM clumping factor B (ClfB) that binds to fibrinogen and several other extracellular matrix proteins was shown to be important in biofilm formation under calcium-depleted conditions [72]. ClfB was recently implicated in facilitating attachment of *S. aureus* to the stratum corneum [73]. While the binding activity of ClfB varies among *S. aureus* strains assessed, these studies provide a molecular basis for how *S. aureus* may initiate colonization on AD skin.

Following attachment, the nascent biofilm forms upon accumulation of bacterial cells via intercellular adhesion events, which occur via two primary mechanisms: polysaccharide-and protein-dependent. The biofilm polysaccharide, poly-*N*-acetylglucosamine (PNAG, also called polysaccharide intercellular adhesin, PIA), is produced by the biosynthetic enzymes of the *icaADBC* operon [74–77]. The PNAG polysaccharide has been shown to contribute to staphylococcal biofilm strength under conditions of high shear stress [78]. The alternate mechanism involves bacterial surface proteins, which directly engage one another to allow staphylococcal cells to adhere together in the biofilm. The accumulation-associated protein (Aap) of *S. epidermidis* is the prototypic member of this protein family; Aap contains an N-terminal A domain upstream of multiple tandem B repeats followed by an extended proline/glycine-rich stalk region [79] that terminates in an LPXTG sortase motif that is covalently attached to the cell wall peptidoglycan [80]. The *S. aureus* ortholog SasG adopts nearly the same domain arrangement. Proteolytic processing of Aap or SasG removes the A domain, unmasking the B-repeat region, which then allows formation of a protein-dependent biofilm, even in the absence of PNAG [81–83]. The B-repeat superdomain of Aap self-assembles in the presence of Zn^{2+} ions to form twisted, rope-like filaments between staphylococcal cells [84–88]; similar Zn^{2+}-dependent assembly has been demonstrated for SasG B repeats [89,

90•]. *S. epidermidis* strains isolated from AD skin contained both the *ica* operon and *aap* gene [14••]. More work will be needed to assess the relative importance of PNAG-dependent versus Aap/SasG-dependent biofilm formation among AD-isolated staphylococcal strains.

Mixed Biofilms

Multi-species biofilms are also prevalent; in fact, the majority of microbes in nature likely exist as members of polymicrobial communities [91]. Such mixed-species biofilms represent an interwoven community of organisms with even more complex interactions [92–94]. There are many examples in which bacterial or fungal species synergize by forming cooperative multi-species biofilms, such as the interactions of *Candida albicans* with *Streptococcus gordonii* in the oral cavity, or *C. albicans* with *S. aureus* in denture stomatitis infections [91]. In some cases, specific interactions between heterologous macromolecules are known to facilitate the inter-species cooperation, as in the case of *C. albicans* protein Als3 directly binding to *Streptococcus gordonii* surface protein SspB [95]. Likewise, the staphylococcal biofilm adhesion proteins Aap and SasG have been shown to form heterophilic assemblies, suggesting that these two staphylococcal species might be able to form mixed biofilms [90•]. Indeed, a recent paper demonstrated that *S. aureus* and *S. epidermidis* can form mixed biofilms in vitro [96], and at least one example has been published of an infected prosthetic joint that was colonized by a mixed *S. aureus* and *S. epidermidis* biofilm [97]. Given the prevalence of both *S. aureus* and *S. epidermidis* in AD skin, it is interesting to speculate that such mixed staphylococcal biofilms may play an important role in the pathogenesis of AD.

Control of Staphylococcal Colonization of Skin by Antimicrobial Peptides

Antimicrobial peptides (AMPs) play a key role in cutaneous immunity [25, 98–100] and are secreted by keratinocytes. AMPs can be constitutively active, while others are induced by infection to combat microbes. Key inducible keratinocyte AMPs are human β-defensin 2, β-defensin 3, and cathelicidin (LL-37), which exert their antimicrobial effect by disrupting bacterial cell membranes. These three AMPs have anti-staphylococcal activity and are strongly induced in psoriasis, an inflammatory skin condition. The levels of these AMPs are much lower in AD due to the presence of Th2 cytokines that downregulate AMP expression [99, 101, 102]. AMPs are also important in modulating innate and adaptive immunity, as they can recruit and activate innate and adaptive immune cells [98]. *S. epidermidis* and other coagulase-negative staphylococci (CoNS) of the skin microbiota can also modulate antimicrobial responses in the skin both directly and indirectly. Certain strains of commensal CoNS provide protection from *S. aureus* colonization through the direct production of staphylococcal AMPs [7], secretion of lipopeptides that stimulate the release of β-defensin from keratinocytes [8], and the induction of immune cell recruitment via IL-1 and IL-17 secreted from macrophages [40]. The commensal staphylococcal AMPs target *S. aureus* to inhibit colonization and can act synergistically with keratinocyte-expressed AMPs [98, 103••]. Interestingly, the CoNS strains that expressed AMPs were found to frequently colonize normal skin but were rarely detected on AD lesional skin [103••]. Furthermore, AD skin also exhibits decreased levels of keratinocyte-secreted AMPs [26, 104], which is correlated with increased *S. aureus* colonization [103••]. Recently, it was shown that the human AMP LL-37 when combined with antimicrobial peptides produced by the

commensal *Staphylococcus hominis* can inhibit *S. aureus* survival more effectively than human or bacterial AMPs individually. Furthermore, restoring strains of *S. epidermidis* or *S. hominis* that inhibited *S. aureus* growth to the skin of two AD subjects led to significant decreases in *S. aureus* colonization compared to vehicle alone. These findings suggest that interactions between microbial communities in the skin play a central role in the pathogenesis of AD and that restoration of antimicrobial commensal strains can be an effective way to control *S. aureus* colonization [103••].

The Impact of Staphylococcal Biofilms on Immune Responses and Keratinocyte Function

A number of studies have begun to assess the specific roles that staphylococcal biofilms play in the pathogenesis of AD. As mentioned, by virtue of growing the biofilm, staphylococci become much more resistant to antibiotic action and immune responses such as phagocytosis. Phagocytosis effectively kills planktonic bacteria and sets the stage for adaptive immune responses [57, 105•]. The formation of a biofilm provides protection to the bacteria within by shielding them from innate immune cells, especially macrophages and neutrophils. Studies have shown that neutrophils are inhibited by *S. aureus* via neutrophilic lysins such as alpha toxin, which is upregulated upon *S. aureus* biofilm formation following neutrophil exposure [106]. Macrophages can either be classically activated in order to present antigen and defend against intracellular pathogens, or these cells can undergo “alternate” activation which is crucial in wound healing and contributes to bacterial persistence [57, 107]. The alternate activation of macrophages contributes to chronicity of these infections, which could be important in disease processes such as AD [106]. Biofilms offer protection from macrophage phagocytosis through several mechanisms. The sheer size of biofilms and the density of the extracellular biofilm matrix have been suggested to render them resistant to engulfment—referred to as “frustrated phagocytosis” [105•, 108]. In addition, macrophage phagocytosis is inhibited by specific proteins secreted from *S. aureus* biofilms, later identified to be alpha toxin, LukA, and LukB. Increased macrophage cytotoxicity was also observed in the presence of *S. aureus* biofilms. *S. epidermidis* biofilms containing increased levels of dormant bacteria led to decreased activation of murine macrophages and less secretion of inflammatory cytokines, suggesting that biofilms aid in immune evasion [109].

In addition to the immune evasion properties mediated by biofilms that lead to recurrent, hard-to-treat infections, staphylococcal biofilms exert direct effects on keratinocytes. For example, a potentially significant impact of *S. aureus* in AD patients is its ability to trigger apoptosis in keratinocytes. Keratinocytes exposed to *S. aureus* biofilms were shown to lose viability and undergo apoptosis after only 3 h of exposure, while those exposed to planktonic culture at 3 h were not statistically different from the control group of keratinocytes alone. Cell morphology was also consistent with keratinocyte apoptosis [110•], although the mechanism for apoptosis was not investigated. This is of importance as damage to epithelial cells releases dsRNA, initiating TLR-3-mediated secretion of thymic stromal lymphopoietin (TSLP) [111]. TSLP secretion results in a strong itch response [112] that can exacerbate excoriation of the skin. Furthermore, TSLP induces dermal dendritic cell activation and

recruitment of Th2 cells that secrete IL-4 and IL-13, which have a suppressive effect on AMPs [26], further limiting protection from pathogens. It was also recently shown that extracts of *S. aureus* biofilms inhibited the terminal differentiation of keratinocytes [113•]. The biofilm extracts induced secretion of IL-6 from the keratinocytes, leading to a decrease in expression of the important differentiation markers keratin 1 and 10, as well as filaggrin (Fig. 3). Furthermore, this block of terminal differentiation renders the keratinocytes more susceptible to the cytotoxic effects of staphylococcal alpha toxin, which was shown to be secreted by *S. aureus* biofilms grown on reconstructed human epidermal tissue [114].

Clinical Implications

Treatment of AD focuses on preventing or reducing bacterial colonization in lesions and controlling inflammation using moisturizers and topical corticosteroids [1]. Bleach baths are also used to reduce bacterial load present on the skin, and recently, these have been shown to inhibit *S. aureus* biofilm formation and reverse pre-formed *S. aureus* biofilms [115]. However, when experiments were repeated on skin biopsies from patients with AD, a 0.16% sodium hypochlorite solution was needed to eradicate 90% of the bacteria present on the biopsy, whereas only 0.005–0.01% sodium hypochlorite solutions were tested on keratinocytes for toxicity [115]. Further studies will be needed to assess the effects of higher amounts of sodium hypochlorite on keratinocytes and to explore the possibility of recurrence of bacterial colonization.

As described earlier, AMPs are key to preventing *S. aureus* colonization [98–100, 116, 117]. The isolation of commensal strains that produce protective AMPs has been used to assess the effects of AMP replacement on *S. aureus* colonization. In AD patients, investigators observed a significant difference in *S. aureus* colonization in skin treated with the commensal bacteria versus vehicle control [103••]. Recent studies have also explored the therapeutic use of human keratinocyte AMPs in treating biofilms; LL-37 was able to eradicate pre-existing MRSA biofilms in a wounded skin model without compromising keratinocyte function [56, 118]. However, additional in vivo studies are needed to determine if replenishing AMPs is an effective treatment option.

As dysbiosis is a driving force in AD, restoration of balance in microbial communities is a target of upcoming treatments. Recently, commensal skin bacteria from healthy human individuals have been transplanted to the skin of mice with induced AD to re-establish a balanced microbiome [119]. To counterbalance the observed decrease in gram-negative (GN) species in patients with AD, culturable GN strains from AD patients and healthy volunteers were tested for their effects on *S. aureus. Roseomonas mucosa* isolated from healthy volunteers, but not from AD patients, was shown to inhibit *S. aureus* growth in vitro. These findings suggest that specific GN strains can exert a bacteriostatic effect on *S. aureus*. When *R. mucosa* strains from healthy volunteers were transplanted onto the skin of mice, the authors observed decreased colonization of *S. aureus*, along with improved transepithelial water loss measurements and decreased redness and swelling in the ears [119]. In future studies, it will be interesting to assess the overall contribution of transplanted individual species or even particular strains on AD outcomes. These methods

can also be applied to other organisms such as *S. epidermidis* or other commensal staphylococcal species.

Conclusion

The severity of AD is significantly influenced by the colonization of *S. aureus and S. epidermidis*, which colonize the skin via microbial communities known as biofilms. Recent studies have reported staphylococcal biofilms colonizing eccrine ducts adjacent to lesional skin in patients with AD, and a number of studies have demonstrated significant impacts of staphylococcal biofilms on the differentiation, apoptosis, or cytokine secretion by keratinocytes. These studies highlight the importance of staphylococcal biofilms in the pathogenesis of AD and highlight the importance of studying host-microbial interactions and their implications for host immunity in AD and allergic disease. Further understanding of *S. aureus* biofilms in the context of AD will allow for development of better treatments to reduce skin colonization, reduce flares, and dampen the rampant Th2 response that likely contributes to the development of additional co-morbidities.

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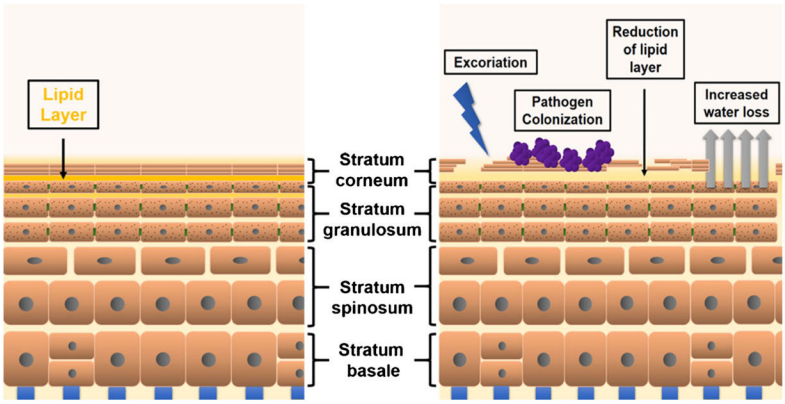

Fig. 1.

Barrier function in healthy and AD skin. Keratinocytes proliferate in the stratum basale and migrate to the stratum granulosum where lipids are secreted into the stratum corneum. The stratum corneum houses keratinocytes that have lost organelles, flatten, and eventually slough off. In AD, increased water loss is a result of a loss of the lipid layer surrounding corneocytes in the inner stratum corneum that acts as a barrier to water-soluble substances. With excoriation, pathogens such as *S. aureus* are able to colonize the skin more readily

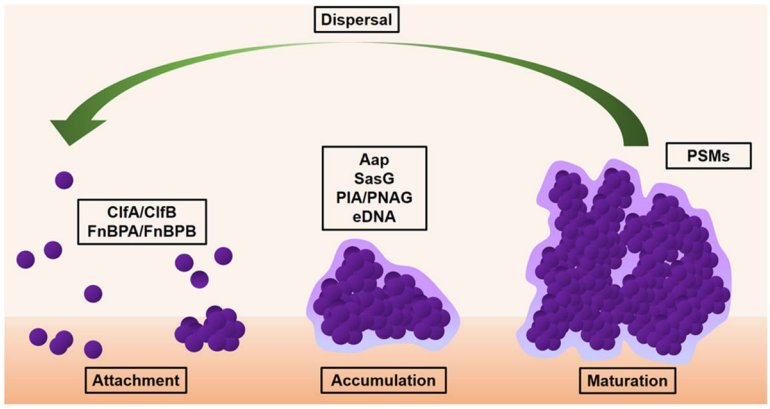

Fig. 2.

Stages of biofilm formation. Bacterial biofilms begin with attachment to a biotic or abiotic surface. Attachment to host tissue typically occurs via MSCRAMM adhesins such as clumping factors (ClfA or ClfB) or fibronectin-binding proteins (FnBPs). Through either polysaccharide (PIA/PNAG) or protein (Aap/SasG) interactions mediating cell-to-cell adhesion, cells begin to accumulate. Remodeling of the biofilm and dispersal of planktonic bacteria is dependent on phenol-soluble modulins (PSMs) under the control of the Agr quorum sensing system

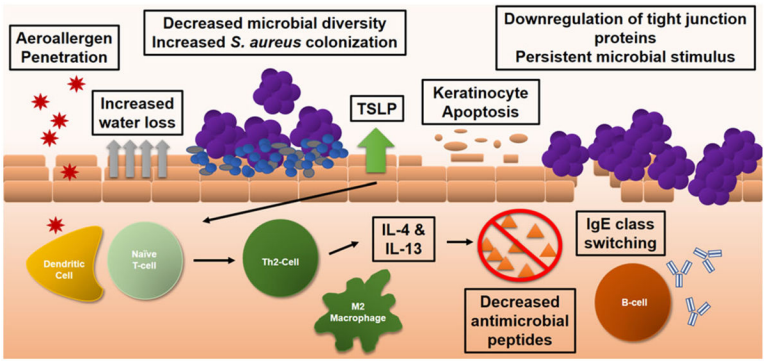

Fig. 3.

Immune dysfunction in atopic dermatitis. Genetic predispositions, such as *FLG* mutations, can weaken the physical strength of the epidermal barrier. In addition, colonization by *S. aureus* also causes inflammation and excoriation, worsening barrier function. With loss of barrier function, aeroallergens are able to interact with dendritic cells, and via antigen presentation, activate naïve T cells. The presence of TSLP enables naïve T cells to differentiate and expand as Th2 cells, which secrete IL-4 and IL-13. These cytokines are important in class switching of B cells to secrete IgE as well as their ability to diminish the secretion of antimicrobial peptides