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Review Article

Staphylococcus aureus in atopic dermatitis: Strain-specific cell wall proteins and skin immunity

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AD, Atopic dermatitis; Atl, Autolysin; CC, Clonal complex; ClfA, Clumping factor A; CoNS, Coagulase negative staphylococci; FLG, Filaggrin; FnBPs, Fibronectin binding proteins; Hsc70, Heat shock cognate 71 kDa protein; Hsp60, Heat shock protein 60; IL, Interleukin; MSCRAMMs, Microbial surface components recognizing adhesive matrix molecules; *S. aureus*, *Staphylococcus aureus*; SdrD, Serine aspartate repeat containing protein D; *S. epidermidis*, *Staphylococcus epidermidis*; SE, Staphylococcal enterotoxin; TLR, Toll-like receptor; TNFR1, Tumor necrosis factor receptor-1; TSST-1, Toxic shock syndrome toxin-1

ABSTRACT

Atopic dermatitis (AD) is a common chronic skin disease. The presence of the bacterium *Staphylococcus aureus* (*S. aureus*) is frequently detected on skin affected with AD. In this review, we focused on the characteristics of *S. aureus* strains isolated from AD skin, particularly the proteins on the cell surface that modulates the interactions between Langerhans cell, keratinocyte, and *S. aureus*. The skin microbiome plays an important role in maintaining homeostasis of the skin, and colonization of *S. aureus* in AD is considered to be deeply involved in the clinical manifestation and pathogenesis of skin flares. Colonizing *S. aureus* strains in AD harbor different surface proteins at the strain level, which are indicated as clonal complexes. Moreover, the cell wall proteins of *S. aureus* affect skin adhesion and induce altered immune responses. *S. aureus* from AD skin (AD strain) exhibits internalization into keratinocytes and induces imbalanced Th1/Th2 adaptive immune responses via Langerhans cells. AD strain-derived cell wall proteins and secreted virulence factors are expected to represent therapeutic targets. In addition, the microbiome on the AD skin surface is associated with skin immunity; thus, microbiome-based immunotherapy, whose mechanism of action completely differs from that of typical steroid ointments, are expected to be developed in the future.

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Introduction

Atopic dermatitis (AD) is an inflammatory dermatosis characterized by repeated chronic eczema, and it is a common disease worldwide.¹ While there are regional differences in the prevalence of AD, it has been reported at about 20% for children (under the age

of 12 years) and 7%–10% for adults.^{2,3} In regard to the pathology of AD, the following three conditions are considered key factors: (1) abnormal skin barrier function, (2) cutaneous immune abnormality predisposed to Th2 immunity, and (3) decreased diversity of bacterial flora on the skin surface (dysbiosis).

In skin barrier dysfunction, about 10% of patients with AD are known to have filaggrin (*FLG*) mutation with increased trans-epidermal water loss.⁴ Epidemiological studies have shown that the development of AD was reduced by treating the impaired skin barrier with moisturizer during the neonatal period.⁵ These studies suggest that skin provides a physical barrier to reduce AD development. In regard to cutaneous immunity, it has been reported that

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Th2 and Th22 cytokines were increased in the lesional skin of patients with AD.⁶ Th2 immunity is believed to be a key feature in impaired cutaneous immunity in AD. In support of this concept, dupilumab, the first biologic for the treatment of AD via inhibition of the Th2 signal by blocking the interleukin (IL)-4 receptor, has been shown to ameliorate dermatitis.⁷ In addition to Th2-shifted immune abnormality and skin barrier impairment, the impact of the skin microbiome on the immune system has been highlighted.⁸ The importance of the skin microbiome was shown in a murine model by applying specific microbiota to the skin surface and changing the cytokine environment within the skin.⁹ Moreover, AD-like dermatitis was induced experimentally by shifting dysbiosis via the application of *Staphylococcus aureus* (*S. aureus*).¹⁰

The skin is an external barrier between the body and the environment that maintains homeostasis by scanning for invaders from the outside. The detailed mechanisms underlying the maintenance of skin homeostasis by the microbiome needs to be fully elucidated. In this review, we focus on the skin microbiome and outline the specific strains of *S. aureus*, isolated from AD skin, that are known to affect the immune system of patients with AD.

The role of *S. aureus* in AD skin

S. aureus is frequently detected in patients with AD; a recent meta-analysis of 95 reports showed that *S. aureus* was detected in about 70% of AD patients with lesional skin and in 39% of AD patients with non-lesional skin.¹¹ In contrast, *S. aureus* is rarely detected in healthy skin.¹² Similarly, analysis of the skin microbiome using the next-generation sequencing revealed that *S. aureus* was detected in patients with AD, and its abundance of the bacterium greatly increased during a skin flare.¹³ Epidemiological surveillance showed that impetigo contagiosa normally caused by *S. aureus* was significantly more frequent in patients with AD.¹⁴ Furthermore, the density of *S. aureus* correlated with the severity of AD,¹⁵ and distribution of *S. aureus* on the body surface is also known to be different.¹⁶ This non-uniform colonization of *S. aureus* on AD skin may be related to the particular distribution of

dermatitis (e.g., on the face and flexor aspect of the extremities) seen in patients with AD. These microbiome analyses and epidemiological data suggest that *S. aureus* is deeply involved in the pathology of AD.

The colonization of *S. aureus* even in non-lesional skin of patients with AD¹¹ and induction of AD-like dermatitis by dysbiosis with *S. aureus*¹⁰ support the notion that the presence of *S. aureus* leads to inflammation of the skin. In addition, the shift in dominant *S. aureus* microbiota during an AD flare¹³ suggest that skin inflammation accelerate the colonization of *S. aureus*. Therefore, the colonization of AD skin by *S. aureus* and skin inflammation can synergistically induce worsening of AD; however, the detailed mechanisms underlying *S. aureus* colonization and AD exacerbation have not been sufficiently elucidated.

In contrast to the extensive colonization of *S. aureus* on AD skin, *Staphylococcus epidermidis* (*S. epidermidis*) is abundant on healthy skin; further, the establishment of non-*S. aureus* colonization at 2 months of age decreases the risk of developing AD.¹⁷ Several reports have focused on the immune responses to *S. epidermidis* compared with those to *S. aureus*.^{18,19} Secretion of IL-10 with activation of regulatory T cells was induced by monocyte-derived dendritic cells stimulated by supernatant from *S. epidermidis*, whereas this effect has not been observed for *S. aureus*.¹⁸ Furthermore, lipoteichoic acid derived from *S. epidermidis* and *S. aureus* elicited divergent immune profile via dendritic cells, with *S. epidermidis* stimulating the production of anti-inflammatory cytokines.¹⁹ Based on these findings, in contrast to *S. aureus*, *S. epidermidis* induces immune tolerance and is presumed to contribute to the maintenance of skin homeostasis.

AD-specific *S. aureus* strains

In addition to the differences between the strains of *S. aureus* and *S. epidermidis*, altered skin immune responses induced by AD skin-derived strains of *S. aureus* have been previously demonstrated by us^{20,21} (Fig. 1). Comparison of laboratory strains of *S. aureus* (standard strain) and clinical isolates from AD skin (AD

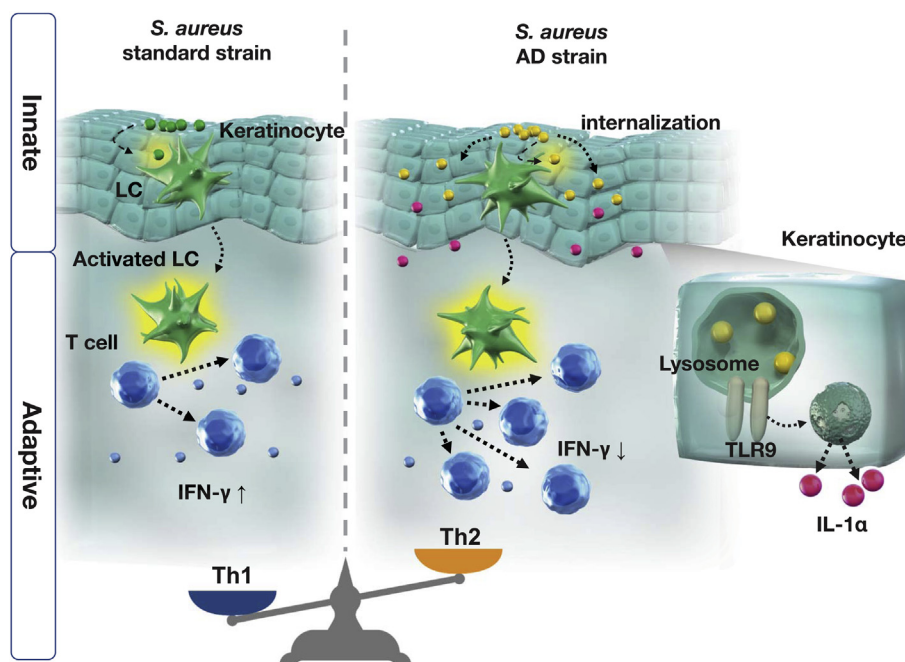


Fig. 1. Graphical summary of skin immune responses induced by *S. aureus* from AD skin (AD strain) and the standard strain. The AD strain elicits typical innate and adaptive immune responses that exacerbate AD. The AD strain stimulates Langerhans cells inducing T cell proliferation with imbalanced Th1/Th2. The AD strain is efficiently internalized into keratinocytes with IL-1 α production via TLR9 stimulation. AD, Atopic dermatitis; *S. aureus*, *Staphylococcus aureus*; IL, interleukin; TLR9, Toll-like receptor 9.

strain) revealed that the AD strain alters the T cell responses via Langerhans cells, resulting in Th2-shifted immune responses. However, *S. epidermidis* elicited mostly the same responses as the standard strain.²⁰ Therefore, in addition to the genetic background of patients with AD, these findings suggest that the colonized AD strain on the skin surface itself may promote an AD-specific immune environment. In line with our previous report,²⁰ Byrd *et al.* reported that *S. aureus* strains isolated from patients with severe AD caused higher levels of skin inflammation.²² Therefore, among *S. aureus*, the importance of skin-disease related strains has been recognized as a particular area of interest.

In addition to the altered Th1/Th2 adaptive immune response elicited by the AD strain, exploration of the innate immune responses involving keratinocytes showed that the AD strain was specifically internalized into lysosomes of the keratinocytes, indicating divergent morphological behavior. Furthermore, it was found that accumulation of the AD strain in lysosomes induced IL-1 α production via the Toll-like receptor 9 (TLR9).²¹ However, effects on pathology and skin colonization due to uptake of the AD strain into keratinocytes have not been elucidated to date. Recent research has demonstrated that activation of complement on the cell surface in keratinocytes incorporating *S. aureus* functions to maintain tissue homeostasis. Interestingly, complement deposition on keratinocytes was found in the skin of patients with AD.²³ Compared with the standard strain, AD strains appear to elicit a specific immune response, and the specificity of the AD strain was maintained even if *S. aureus* was immobilized.²⁴

Recent progress in identifying *S. aureus* at the genetic level (clonal complex [CC]) revealed that AD skin harbors a specific strain of *S. aureus*^{25–29} (Table 1). The CC type is identified by DNA sequencing of *S. aureus*-specific staphylococcal protein A (*spa* typing). However, the virulent factors may be expressed differently in various clonal lineage, and studies analyzing the clinical associations between them are limited. CC 30 is reported to be associated with endocarditis,³⁰ but clinical lineage relative to AD is still unclear, although country-specific surveys for CC typing have been performed recently. In the United Kingdom, CC1 was detected more frequently in patients with AD, whereas CC30 was more common in healthy subjects.²⁵ A similar tendency was found in several countries such as Ireland²⁶ and Spain,²⁷ and the existence of *S. aureus* strains colonized in AD skin is becoming apparent. In addition, it has been reported that CC1 is frequently detected on AD skin of individuals with the *FLG* gene mutation,²⁸ and the predisposing factors to AD also promote the colonization of AD strains on the skin surface. Further analyses of *S. aureus* worldwide are needed to classify the characteristics of AD strains, leading to the discovery of the distinct surface or genomic markers of AD-derived *S. aureus*.

Virulence factors from the surface proteins of *S. aureus*

In regard to the pathogenicity of *S. aureus*, secreted exotoxins and surface proteins present on the surface layer of *S. aureus* are known as virulence factors.^{31,32} Toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxin (SE) and exfoliative toxin are known superantigens involved in skin infection.³³ Clinical isolates from AD skin are tend to secrete higher amounts of toxins such as TSST-1 and SE.³⁴ It has been reported that the application of SE to the skin caused activation of T cells³⁵ and elicited AD-like eruption in a mouse model.³⁶

Expression of the cell wall proteins of *S. aureus* increases during the growth phase of bacteria and is involved in their colonization and skin immune responses³¹ (Fig. 2). In infectious diseases, the expression of cell wall proteins of *S. aureus* increases and is involved in the aggravation of infection,³⁷ whereas sortase-deleted *S. aureus*, which loses covalently binding cell wall proteins, is less able to develop abscess formation.³⁸ In line with these findings, we demonstrated that the specific immune responses of *S. aureus* derived from AD skin were impaired by removing cell wall proteins of AD strains via proteinase K treatment; this was verified as decreased activation of Langerhans cells²⁰ (Fig. 3A) and diminished internalization of *S. aureus* into keratinocytes²¹ (Fig. 3B). In addition to exotoxins, cell wall proteins of disease-derived *S. aureus* strains are able to shape AD-specific immune reactions.

Several kinds of cell wall proteins have been well characterized (Fig. 2), and their functional roles in adhesion to epithelial cells and abscess formation have been reported.³⁹ Microbial surface components that recognize adhesive matrix molecules (MSCRAMMs) expressed on the surface of *S. aureus* play a key role in the adherence of *S. aureus* to the cells.⁴⁰ More than 20 proteins classified as MSCRAMMs covalently anchored by sortase to peptidoglycan, such as fibronectin-binding proteins (FnBPs), have been identified.³⁹ During the internalization of *S. aureus* into epithelial cells, FnBPs can bind to integrin $\alpha 5 \beta 1$ of epithelial cells via fibronectin. This pathway represents the major mechanism involved in the adhesion and uptake of *S. aureus* into keratinocytes.⁴¹ Interestingly, fibronectin is strongly expressed in the upper layer of the epidermis of AD skin relative to that in healthy skin, suggesting that this protein promotes colonization of *S. aureus* in AD skin.⁴² Accordingly, we have demonstrated that internalization of the AD strain is decreased by blocking fibronectin,²¹ and suggested that the binding of FnBPs to integrin is the main route for internalization of *S. aureus*. However, the internalization of *S. aureus* was not completely inhibited only by blocking this binding. Thus, other receptors for internalization such as heat shock protein 60 (Hsp60) may also be involved in this process (Fig. 2).⁴³

Table 1

Summary of the clonal complex of *S. aureus* isolated from AD skin.

Country Year	UK ²⁵ 2018		Ireland ²⁶ 2017		Spain ²⁷ 2014		Denmark ²⁸ 2017	Canada ²⁹ 2010
Clonal complex	HC (n = 18)	AD (n = 9)	HC (n = 49) [†]	AD (n = 44) [†]	Atopic Control (n = 31)	AD (n = 32)	AD (n = 74)	AD (n = 160)
CC1	6	44	4	21	3	3	22	11
CC5	6	11	15–20	14	10	31	10	15
CC8	0	0	2	14	3	6	5	11
CC15	17	0	2	7	3	19	19	14
CC30	61	11	33	7	48	19	7	7
CC45	6	11	20–25	16	10	16	15	21
CC59	0	11	0	5	—	—	3	—
CC121	0	11	2	2	—	—	0	—

Reported clonal complex and defining strain from European countries and North America represent the typical characteristics of *S. aureus* populations in AD skin. The table was generated from references 25 to 29. Frequency was indicated by %.

[†] Collected number of strains. AD, Atopic dermatitis; *S. aureus*, *Staphylococcus aureus*.

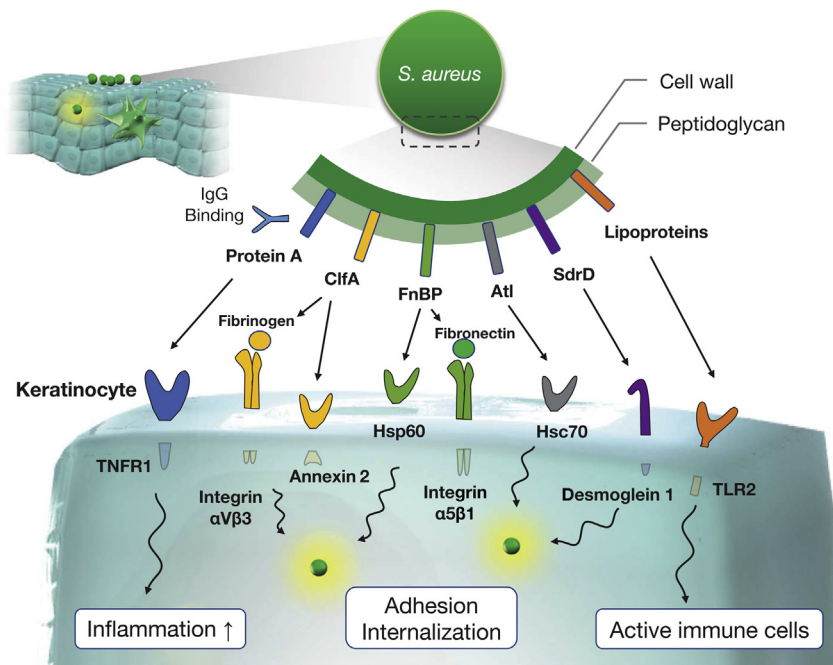
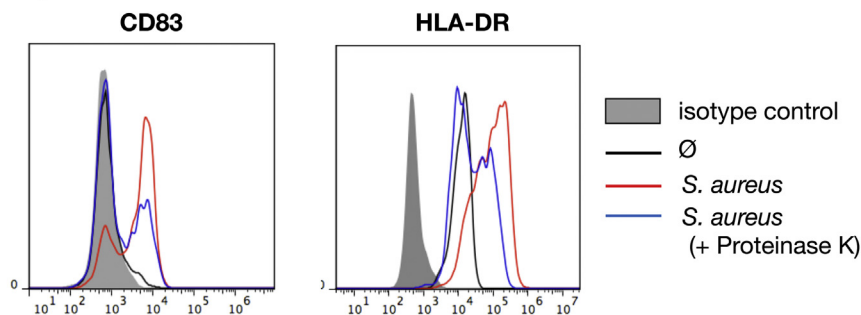


Fig. 2. Cell wall proteins of *S. aureus* interacting with keratinocytes. Cell wall proteins of *S. aureus* (ClfA, FnBP, Atl, and SdrD) are involved in adhesion and internalization into keratinocytes via respective receptors (Annexin2, Hsp69, Integrin α 5 β 1 with fibronectin bridging, Hsc70, and Desmoglein1). Protein A induces inflammation by binding to TNFR1, and lipoprotein is sensed by TLR2 following activation of immune cells. ClfA, Clumping factor A; FnBP, Fibronectin binding proteins; Atl, Autolysin; SdrD, Serine aspartate repeat containing protein D; *S. aureus*, *Staphylococcus aureus*; Hsp60, Heat shock protein 60; Hsc70, Heat shock cognate 71 kDa protein; TNFR1, Tumor necrosis factor receptor-1; TLR2, Toll-like receptor 2.

Clumping factor A (ClfA) is a surface protein known to be able to adhere to host cells via two different receptors, namely integrin α V β 3 expressed on the host cell via fibrinogen as a bridge,⁴⁴ and

annexin 2, which ClfA binds directly.⁴⁵ ClfA is an important virulence factor that contributes to infection.³⁹ ClfA-deficient *S. aureus* demonstrates a lower bacterial burden in skin abscesses compared

A. Langerhans cell



B. Keratinocyte

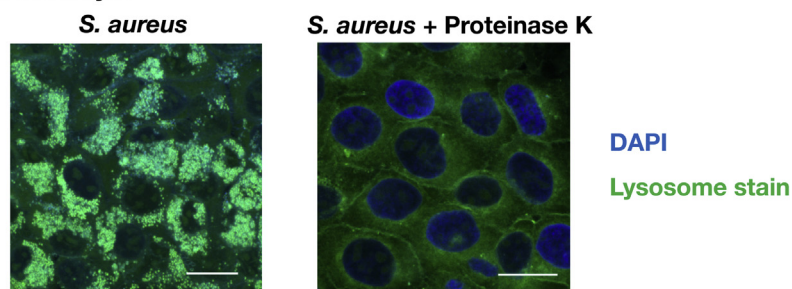


Fig. 3. Removal of cell wall proteins from *S. aureus* (AD strain) by proteinase K diminishes their immune activities. **A)** Flow cytometry analysis shows decreased expression of CD83 and HLA-DR, an activation marker of Langerhans cells, after treating *S. aureus* with proteinase K. **B)** Internalization of *S. aureus* into keratinocytes is decreased by treating *S. aureus* with proteinase K. Scale bar, 20 μ m ($\times 120$). Data adapted from references 20 and 21. AD, Atopic dermatitis; *S. aureus*, *Staphylococcus aureus*; HLA-DR, Human leukocyte antigen-DR; DAPI, 4',6-diamidino-2-phenylindole.

with wild-type strains.³⁸ Decreased binding of ClfA-negative *S. aureus* strain to AD skin has been observed; however, adherence of this mutant strain to the psoriatic and normal skin did not change.⁴² In particular, ClfA may be involved in the colonization of skin with AD, but not of healthy skin or skin with other inflammatory conditions. ClfA may also serve as an antigen to stimulate T cells, inducing Th1 cytokine secretion.⁴⁶ SdrD (Serine aspartate repeat containing protein D) is another MSCRAMM that has been demonstrated to adhere via desmoglein 1 expressed on keratinocytes.⁴⁷ Atl (Autolysin) is a non-covalently bound protein known to play an important role in cell proliferation and segregation, and it adheres to the host cell via Hsc70 (Heat shock cognate 71 kDa protein), triggering internalization of *S. aureus*.⁴⁸

Lipoteichoic acid serves as a ligand for TLR2 and induces inflammation.⁴⁹ TLR2-mediated activation functions as a biological defense against microbes, as shown using TLR2 knockout mice.⁵⁰ As expected, AD skin possessed a bioactive amount of lipoteichoic acid, another TLR2 agonist from *S. aureus* that is detected in the lavage fluid of skin lesions of patients with AD.⁵¹ Analysis using human AD skin colonized by *S. aureus* revealed that Langerhans cells, the “control tower” for skin immunity, expressed lower TLR2 levels in AD skin than in healthy skin. Even with continuous stimulation of TLR2 by colonized AD strains, the responsiveness of Langerhans cells and subsequent T cell responses were tolerated.⁵² Protein A is the main antigen among cell wall proteins that binds to immunoglobulins, enabling escape from the host defense. TNFR-1 (tumor necrosis factor receptor-1) is the receptor for protein A that causes inflammation⁵³; most *S. aureus* strains isolated from AD skin express protein A, as confirmed by biopsy of tissues from patients with AD.^{54,55} Interestingly, protein A was identified even in the intercellular space of keratinocytes by immunoelectron microscopy,⁵⁵ suggesting that *S. aureus* colonizing AD skin is internalized with protein A and causes local skin inflammation.

During colonization of skin by *S. aureus*, AD strains harboring abundant FnBPs²¹ tend to adhere to AD skin. In addition, the characteristic Th2 immune environment in AD accelerates the colonization of *S. aureus* via increased fibronectin production in the skin.⁵⁶ Moreover, the proliferation of *S. aureus* is inhibited under FLG degradation products, suggesting that AD patients with FLG mutation are likely to exhibit increased susceptibility to colonization by *S. aureus*.⁵⁷

Development of treatments targeting *S. aureus*

Current treatments for AD that involve the use of topical steroids improve dermatitis owing to their strong anti-inflammatory effect. However, such treatments are associated with side effects such as skin thinning with long-term use and recurrence of dermatitis after discontinuation. To compensate for the limitations of conventional topical steroid treatment, several attempts have been made to achieve the removal of colonized *S. aureus*, by targeting strain-specific characteristics.

Bleach bath therapy for sterilization of *S. aureus* is applied as the treatment for AD, and the effectiveness of sodium hypochlorite baths has been reported in a randomized, double-blinded study.⁵⁸ However, a bleach bath, which reduced topical corticosteroid use and topical antibiotic use, was not found to be more effective than a water bath.⁵⁹ Further studies will be necessary to determine the appropriate indication for bleach bath therapy in patients with AD. In recent years, studies have focused on achieving a balanced skin microbiome, rather than removing *S. aureus*. Antimicrobial peptides produced from coagulase-negative staphylococci (CoNS), such as *S. epidermidis* and *Staphylococcus hominis*, are able to eradicate *S. aureus*. Indeed, applying these CoNS strains to the skin of patients with AD has been shown to decrease the abundance of

S. aureus on the skin surface.⁶⁰ Furthermore, it has been reported that transplantation of the germ-negative bacterium *Roseomonas mucosa* derived from healthy human skin to AD patients improved dermatitis in the latter.⁶¹ In addition, the therapeutic effect of a compound that regulates the agr quorum-sensing mechanism and suppresses the production of the γ -toxin, a secretory exotoxin of *S. aureus*, has been confirmed in a mouse model of AD.⁶²

AD patients with *S. aureus* colonization showed a higher Th2 biomarker level than AD patients without *S. aureus* colonization.⁶³ The IL-4 receptor blocker dupilumab inhibits the subcutaneous Th2 inflammatory response pathway, which may prevent the establishment of *S. aureus*. New systemic immunomodulatory biologics are expected in the foreseeable future,² and it is noteworthy that these therapies will target not only skin immunity but also *S. aureus* colonization by modulating their cell wall proteins of this bacterium.

Conclusions

The pathogenicity of *S. aureus*, especially the relationship between cell wall proteins and skin immunity, was outlined in this review. In regard to skin immunity, the characteristics of AD-derived *S. aureus* strains differ from those of standard *S. aureus* strains. AD strain-derived cell wall proteins and secreted virulence factors are expected to represent a therapeutic target in the development of future treatments. However, several questions, such as how these virulence factors are regulated under an AD Th2-shifted environment and how treatments such as topical steroid ointment and biologics affect the expression of these virulence factors, remain to be resolved. The therapeutic effect of the removal of skin-colonizing *S. aureus* remains to be examined. The microbiome on the AD skin surface is associated not only with aspects of allergic hypersensitivity, but also with skin immunity.

Thus, microbiome-based immunotherapy, whose mechanism of action is completely different from that of topical steroid ointment, is expected to provide the foundation for the development of new strategies to treat AD.

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Conflict of interest

The authors have no conflict of interest to declare.

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