Staphylococcus aureus Exploits Epidermal Barrier Defects in Atopic Dermatitis to Trigger Cytokine Expression

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Patients with atopic dermatitis (AD) have an abnormal skin barrier and are frequently colonized by S. aureus. In this study we investigated if S. aureus penetrates the epidermal barrier of subjects with AD and sought to understand the mechanism and functional significance of this entry. S. aureus was observed to be more abundant in the dermis of lesional skin from AD patients. Bacterial entry past the epidermis was observed in cultured human skin equivalents and in mice but was found to be increased in the skin of cathelicidin knockout and ovalbumin-sensitized filaggrin mutant mice. S. aureus penetration through the epidermis was dependent on bacterial viability and protease activity, because killed bacteria and a protease-null mutant strain of S. aureus were unable to penetrate. Entry of S. aureus directly correlated with increased expression of IL-4, IL-13, IL-22, thymic stromal lymphopoietin, and other cytokines associated with AD and with decreased expression of cathelicidin. These data illustrate how abnormalities of the epidermal barrier in AD can alter the balance of inflammatory cytokines and exacerbation of disease.


INTRODUCTION

The microbial community can have both beneficial and detrimental functions (Gallo and Nakatsuji, 2011). For example, Staphylococcus epidermidis, a predominant resident on healthy human skin, can suppress inflammation after skin injury, maintain immune tolerance to commensals, modify cutaneous T-cell development, and enhance innate immune defense by inducing expression of antimicrobial peptides (AMPs) (Cogen et al., 2010; Lai et al., 2010; Li et al., 2013; Naik et al., 2015; Naik et al., 2012; Scharschmidt et al., 2015; Wang et al., 2012; Wanke et al., 2011). Conversely, imbalance of the microbiome (dysbiosis) appears to contribute to the pathogenesis of some skin diseases. Strong associations have been shown between dysbiosis and the clinical phenotype of atopic dermatitis (AD) (Leung and Guttman-Yassky, 2014). For example, AD subjects are well known to have increased colonization by Staphylococcus aureus (Leyden et al., 1974) and a loss in bacterial diversity on the skin (Kong et al., 2012). Furthermore, recent mechanistic studies have shown that S. aureus can drive development of AD-like lesions in mice (Kobayashi et al., 2015).

These findings suggest that a better understanding of how bacteria influence skin immunity may provide important clues to improve management of AD.

S. aureus can cause inflammation by inducing T-cell-independent B-cell expansion, initiating the production of proinflammatory cytokines such as thymic stromal lymphopoietin from keratinocytes, and stimulating mast cell degranulation, resulting in T helper (Th) 2 skewing (Bekeredjian-Ding et al., 2007; Nakamura et al., 2013; Vu et al., 2010). S. aureus also disrupts proteolytic balance in the skin by inducing multiple metalloproteases in dermal fibroblasts (Kanangat et al., 2006). However, because of the complex structures and cell networks that make up mammalian skin, the mechanism by which S. aureus disrupts cutaneous inflammatory homeostasis is incompletely understood. It appears that most beneficial and detrimental actions of skin bacteria are dependent on their capacity to interact with host cells that reside under the surface stratum corneum. Until recently it was unclear how skin surface microbes could influence immunological responses through a stratum corneum structure.

We recently observed that bacteria can be observed within the dermis of healthy normal human skin (Nakatsuji et al., 2013). This surprising observation that bacteria can penetrate the epidermis illustrated how bacteria position themselves to directly influence immune responses. The epidermis apparently acts as a regulator of microbiome entry rather than as an absolute barrier to microbes. This suggests that epidermal barrier defects such as the loss-of-function mutations found within the filaggrin gene (FLG) (Bisgaard et al., 2008; Palmer et al., 2006; Sandilands et al., 2007; Smith et al., 2006) could promote disease. In addition, the epidermis of AD subjects can have a decreased capacity to...
produce AMPs such as cathelicidin and β-defensins (Hata et al., 2010; Howell et al., 2006a; Howell et al., 2006b; Mallbris et al., 2010; Ong et al., 2002). Such antimicrobial or physical barrier defects may facilitate physical penetration of the epidermis by bacteria that otherwise would not trigger inflammation on normal skin.

In this study we sought to determine the mechanism by which S. aureus penetrates the epidermis. We hypothesized that the altered physical and antimicrobial barrier of the skin in AD would result in enhanced penetration of S. aureus across the epidermal surface and that this can contribute to the loss of immune homeostasis. Such an interaction between bacteria on the skin surface and cells in the dermis provides a unifying hypothesis to explain why genetic or environmental defects in the skin barrier drive immunologic abnormalities of AD.

**RESULTS**

**Dysbiosis of the bacterial community in the dermis of patients with AD**

To examine the microbial community in the dermis of skin from subjects with AD, skin biopsy samples from control subjects without AD and from lesional and nonlesional sites of AD patients were obtained. Biopsy samples of lesional AD skin did not include skin sites that were excoriated. Skin samples were separated into epidermal and dermal tissues by laser-capture microdissection (LCM) (see Supplementary Figure S1a online), and bacterial DNA contained in each tissue was analyzed by quantitative real-time PCR (qPCR) and pyrosequencing. The absolute abundance of 16S rDNA in the LCM-dissected epidermis and dermis was higher in lesional skin than in non-AD and nonlesional AD skin (Figure 1a). S. aureus was detected by qPCR in both epidermis and dermis of lesional skin but not in non-AD and nonlesional AD skin (Figure 1b). S. epidermidis DNA was also detected in epidermis and dermis of non-AD and AD skin and was higher in dermis of lesional skin than that of non-AD and nonlesional AD skin (Figure 1c). Pyrosequencing for 16S rDNA detected relatively higher abundance of Firmicutes (the phylum of Staphylococcus species) in epidermal and dermal compartments of lesional AD skin than those of nonlesional AD skin (Figure 1d). To detect potential contamination, nontissue controls were prepared from the

![Figure 1. Dysbiosis of the subepidermal compartments from skin of AD patients.](image)

**Figure 1. Dysbiosis of the subepidermal compartments from skin of AD patients.** qPCR results for relative abundance of DNA for (a) 16S rRNA, (b) Staphylococcus aureus, and (c) Staphylococcus epidermidis detected in epidermal or dermal compartments, isolated by LCM, of normal skin of non-AD subjects and nonlesional and lesional skin of patients with AD. NTCs were simultaneously processed. Data represent mean ± standard error of the mean of 11 subjects. *P < 0.05, **P < 0.01. (d) 16S rDNA pyrosequencing results from samples isolated by LCM of the epidermis and dermis of nonlesional and lesional skin of AD subjects. Each bacterial phylum is shown in a different color. (e–g) Immunofluorescence for S. aureus and keratin-14 in (e) lesional or (f) nonlesional skin of an AD subject. (g) Staining with isotype control. (h) Immunofluorescence for S. aureus and CD11c in lesional skin of an AD subject. (i) Staining with isotype control. White arrows indicate S. aureus staining detected outside of CD11c+ immune cells. Immunostaining shown is a representative of three biopsy samples from different donors. Scale bar = 20 μm (white) or 200 μm (yellow). AD, atopic dermatitis; Derm, dermis; Epi, epidermis; L, lesional; LCM, laser-capture microdissection; N, nonlesional; NTC, nontissue control; qPCR, quantitative real-time PCR; rCFU, relative colony-forming unit; UD, undetectable.
same tissue blocks and assay reagents, and DNA was not detected in these samples (Figure 1a–c).

To further confirm these observations, lesional and nonlesional skin was stained for S. aureus, and this directly showed that S. aureus was more abundant within the epidermis and dermis of lesional skin compared with nonlesional samples (Figure 1e–f). Notably, S. aureus was independently detected in the epidermis and dermis of lesional AD skin and not associated with CD11c+ immune cells (Figure 1h), thus suggesting a penetration mechanism independent from classical phagocytic cells. No immunoreactivity was seen with control IgG (Figure 1g and i).

*S. aureus* penetrates cultured human skin equivalents

To begin to understand how *S. aureus* may enter the dermis, we next compared the capacities of live and dead *S. aureus* to penetrate the epidermis of an organotypic human skin equivalent. Immunohistochemical analysis was done on samples processed at 13, 24, and 48 hours after *S. aureus* application. Immunostaining of the skin equivalent showed that *S. aureus* were present at progressively deeper layers in the epidermis in a time-dependent manner and were detectable below the basement membrane by 48 hours (Figure 2a–c). In contrast, despite application of a larger bacterial load, bacteria that were killed by UV radiation remained on the surface at 48 hours (Figure 2d). These data showed that *S. aureus* must be viable to penetrate the epidermis.

Cathelicidin inhibits bacterial entry into dermis

Because we observed that penetration of skin required viable bacteria, we hypothesized that AMP activity may act to limit bacterial entry into the dermis. To test this, *S. aureus* was applied to dorsal skin of cathelicidin gene (*Camp*) knockout or wild-type (WT) mice. Mouse skin was then separated by LCM into control, epidermal, dermal, and dermal adipose compartments (see Supplementary Figure S1b). After application to the skin surface, *S. aureus* abundance was not statistically different on the epidermis of WT and *Camp*−/− mice (Figure 3a). However, *S. aureus* was significantly more abundant in the dermis and adipose tissue of *Camp*−/− mice than WT mice (Figure 3b–d). These data suggest that the antimicrobial barrier provided by cathelicidin limits entry of *S. aureus* into the skin and is one variable controlling bacterial penetration into the dermis.

*S. aureus* penetration is enhanced in a mouse loss-of-function—of—filaggrin model of AD

We next evaluated entry of *S. aureus* in loss-of-function—of—filaggrin (Flgft/ft) mice (Fallon et al., 2009). Similar to previous reports, Flgft/ft mice developed AD-like skin inflammation after mechanical barrier disruption by tape-stripping and repeated application of ovalbumin (OVA), whereas WT mice or Flgwt/WT without OVA sensitization had much less inflammation (see Supplementary Figure S2 online). Consistent with this observation, the skin of Flgft/ft mice after tape-stripping and OVA sensitization showed enhanced transepidermal water loss compared with WT mice (Figure 4a). Twenty hours after application of *S. aureus* to the skin of these mice there was only a minimal difference in *S. aureus* detected in the surface epidermal compartment of the Flgft/ft mice (Figure 4b), whereas a significantly increased amount of *S. aureus* was detected in the dermis and adipose tissue of Flgft/ft than in WT mice with similar tape-strip and OVA sensitization or phosphate buffered saline-treated Flgwt/WT mice (Figure 4c–d). *S. aureus* was undetectable in nontissue controls (Figure 4e). These data show that in the setting of increased inflammation and enhanced TWEL, a loss-of-function mutation in FLG facilitates bacterial entry through the epidermal barrier.

*S. aureus* proteolytic activity enhances penetration of the epidermal barrier

Because *Staphylococcus* extracellular proteases have been known to interact with the epidermal barrier components, we hypothesized that bacteria enter the dermis through the
action of their proteases. To address this, we compared entry of WT methicillin-resistant \textit{S. aureus} USA300 to an extracellular protease-mutant of this strain that lacks 10 major proteolytic enzymes, including aureolysin metalloprotease, V8 and SspA serine proteases, ScpA and SspB cysteine proteases, and six other serine-like protease homologs (Kolar et al., 2013). We confirmed that the culture supernatant of the mutant strain contained less proteolytic activity than that

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\caption{Cathelicidin inhibits \textit{Staphylococcus aureus} entry into the mouse dermis. (a–c) \textit{S. aureus} (ATCC35556) or vehicle (mock) were loaded in agar disks and applied on dorsal skin of \textit{Camp}^{-/-} or wild-type mice for 20 hours. Skin was then excised, and DNA was extracted from (a) epidermis, (b) dermis, or (c) adipose tissue isolated by laser-capture microdissection. Relative colony forming units of \textit{S. aureus} DNA were determined by quantitative real-time PCR by comparison with a standard of known colony forming units of \textit{S. aureus} (ATCC35556). (d) As negative control, a nontissue control was simultaneously processed with the same reagents from embedding material adjacent to each tissue section. The data were normalized against tissue volume excised by LCM. Data represent mean ± standard error of mean of results from four independent experiments. *P < 0.05. NTC, nontissue control; rCFU, relative colony-forming unit; UD, undetectable; WT, wild type.}
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\caption{A loss-of-function mutation in filaggrin increases \textit{Staphylococcus aureus} entry into the mouse dermis after ovalbumin sensitization. (a) Transepidermal water loss determined before (–) or after (+) tape-stripping on the dorsal skin of Flg^{-/-} Balb/c or WT mice that were treated by repeated applications of OVA or phosphate buffered saline. (b–e) The backs of Flg^{-/-} Balb/c mice, or WT Balb/c mice were treated with tape-stripping and OVA as described in panel a. Abundance of \textit{S. aureus} (ATCC3555) in (b) epidermis, (c) dermis, and (d) adipose tissue was measured by quantitative real-time PCR and laser-capture microdissection, as described in Figure 3. (e) Nontissue control was simultaneously processed as negative control. Data represent mean ± standard error of the mean of results from six independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. OVA, ovalbumin; PBS, phosphate buffered saline; rCFU, relative colony-forming unit; TEWL, transepidermal water loss; UD, undetectable; WT, wild type.}
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of the WT of the same background strain (see Supplementary Figure S3 online). First, as in Figure 2, an equal number of bacteria were applied on the epidermis of cultured human skin equivalents to compare the capacity of each strain to penetrate the epidermis. Both strains grew at the same rate in this model, because after 48 hours, comparable colony-forming units of the live WT and mutant strains were detected in full-thickness biopsy samples of the skin construct (Figure 5a). More WT *S. aureus* was detected in the dermis than the protease-deficient mutant strain. Next, when these bacteria were applied to mice in the AD skin model of *Flg*<sup>ΔH</sup> mice shown in Figure 4, a similar behavior of *S. aureus* was observed. Both bacterial strains were detected in similar quantities in the mouse epidermis (Figure 5b), whereas the only the WT strain showed a significant capacity to enter the dermis and adipose tissue (Figure 5c–e). These data suggest that skin entry by bacteria is, at least in part, facilitated by microbial proteases.

**Entry of *S. aureus* into the dermis triggers immune abnormalities seen in AD skin**

With the observation that WT and protease-deficient strains of *S. aureus* penetrated differently below the epidermis, it became possible to examine if a correlation exists between entry of bacteria into the dermis and an immunological response. The expression of mRNA for inflammatory cytokines (IL-4, IL-13, CXCL2, thymic stromal lymphopoietin, IL-17a, IL-22, and IFNs) *Camp* was therefore measured in each condition. In accordance with the capacity to enter the skin as seen in Figure 5b and c, live WT *S. aureus* induced more expression of IL-4, IL-13, CXCL2, thymic stromal lymphopoietin, IL-17, and IL-22 but not IFNs (Figure 5f–n). Furthermore, similar to observations in human subjects with AD, *S. aureus* entry resulted in suppression of *Camp* expression (Figure 5o). In contrast, *S. aureus* entry was correlated with a slight increase in β-defensins-14 and -4 expression, but the relative expression level of these β-defensins was much lower than that of *Camp*.

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**Figure 5. *Staphylococcus aureus* protease activity is required for penetration of the epidermis and induction of inflammatory cytokines.** (a) Entry of WT or an extracellular protease-null mutant strain of MRSA into organotypic human skin constructs. (b–e) Entry of WT, extracellular protease-null mutant strain and UV-killed WT strain of MRSA into (b) epidermis, (c) dermis, and (d) adipose tissue of *Flg*<sup>ΔH</sup> BALB/c mice sensitized by OVA was tracked as described in Figure 3. (e) NTC was processed as negative control. (f–o) To correlate entry of MRSA strains with cutaneous immune response, gene expression of (f–n) indicated cytokines and (o) indicated AMPs was measured in the same whole skin biopsy samples from panels b–d. To compare relative expression level of each AMP, data are shown as relative to GAPDH expression. Data represent mean ± standard error of the mean of results from 5–6 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001. AMP, antimicrobial peptide; CFU, colony-forming unit; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; IL, interleukin; MRSA, methicillin-resistant *S. aureus*; NTC, nontissue control; rCFU, relative colony-forming unit; TSLP, Thymic stromal lymphopoietin; UD, undetectable; WT wild type.
Application of a barrier repair cream decreases bacterial penetration of skin

An essential element in the therapy of AD is the topical application of moisturizers or barrier repair products. This can result in a significant decrease in inflammation, but the immunological mechanism responsible for this improvement is unclear. To examine if restoration of skin barrier function could benefit the immune response by limiting bacterial entry into the dermis, AD-like skin lesions in OVA-sensitized Flg<sup>−/−</sup> mice were treated with a barrier repair formulation consisting of optimized ceramide-triple lipid mixture (Man et al., 1996; Mao-Qiang et al., 1995) or vehicle. Subsequently, S. aureus was applied to the surface, and entry was tracked with LCM and qPCR, as described previously. Treatment with the ceramide-triple lipid mixture restored barrier function as measured by transepidermal water loss (Figure 6a). After barrier repair, S. aureus penetration decreased into the subepidermal tissues compared with the control skin treated with vehicle (Figure 6b–e). Application of the ceramide-triple lipid mixture did not exert an antimicrobial effect on the S. aureus colonized on the epidermis (Figure 6b). In addition, barrier repair partially limited cytokine induction and failure of Camp induction caused by S. aureus application (Figure 6f–i).

DISCUSSION

Defects in the skin barrier have been associated with the pathogenesis of AD and are frequently associated with colonization by S. aureus, a factor that exacerbates disease (De Benedetto et al., 2011; Leung and Guttman-Yassky, 2014). It was unclear how dysbiosis at the skin surface could trigger inflammation that arises below the stratum corneum. We report herein that S. aureus penetrates the epidermis by a proteolytic mechanism and that failure of the antimicrobial or physical skin barrier of the epidermis enhances entry of S. aureus into the dermis. Entry of bacteria enables them to come into direct contact with viable immunocytes and stimulates production of proinflammatory cytokines. These data directly show how the skin barrier controls the interaction between the microbiome and the cutaneous immune system and illustrate how abnormal penetration of surface microbes can mediate immune dysregulation associated with AD. Because this immune dysregulation further disrupts the barrier function of the skin, this relationship provides an explanation for the chronic nature of inflammation observed in this disorder.

An important variable that influenced microbial entry into the dermis was AMP expression. Cathelicidin exhibits direct antimicrobial action against a wide range of pathogens.
(Dorschner et al., 2001; Gallo et al., 1994; Nizet et al., 2001). In healthy skin, cathelicidin expression is increased upon infection, inflammation, and injury (Gallo and Hooper, 2012; Lai and Gallo, 2009). However, the skin of patients with AD has been shown in some studies to have a decreased capacity to produce an adequate amount of this AMP and β-defensins-2 and -3 (Hata et al., 2010; Howell et al., 2006a; Howell et al., 2006b; Mallbris et al., 2010; Ong et al., 2002). This AMP deficiency may result in an inability of the skin to resist infection by several pathogens, including S. aureus. However, it was not known if the constitutive presence of cathelicidin expressed in healthy skin contributed to antibacterial defense or how this influenced colonization of skin microbes. In this study, cathelicidin was shown to regulate entry of S. aureus. This finding may be clinically relevant when considered with observations that cathelicidin expression is enhanced by vitamin D and improves clinical outcome in AD (Camargo et al., 2014; Hata et al., 2008; Liu et al., 2006; Schaubert et al., 2007; Schaubert et al., 2006). Therefore, although many variables may confound this response, the capacity of vitamin D to enhance the antimicrobial barrier may partially explain reported benefits of vitamin D3 in AD and other allergic disorders (Goetz, 2011; Malley et al., 2009).

Another variable found here to influence penetration of S. aureus was the expression of filaggrin. Filaggrin is a structural protein that is fundamental in the development and maintenance of the physical skin barrier (Sandilands et al., 2009). Loss-of-function mutations in FLG represent a significant genetic factor predisposing the development of AD in some populations (Bisgaard et al., 2008; Palmer et al., 2006; Sandilands et al., 2007; Smith et al., 2006). Two of the most studied mutations (R501X and 2282del4) are common in European populations and result in loss of function (Weidinger et al., 2006). The flaky-tail mouse used here has a naturally occurring single-base-pair deletion (Flg<sup>ft</sup>) that induces a premature stop codon and also results in loss of function (Fallon et al., 2009; Moniaga and Kabashima, 2011). This mouse differs from the original flaky-tail mouse, which has an additional mutation in Tmem79/Matt, a mutation that is also associated with AD in humans (Saunders et al., 2013). In contrast to the double-mutation original flaky-tail mouse, this Flg<sup>ftt</sup> mouse line does not develop spontaneous inflammation (Hoff et al., 2015). However, out data illustrate how mutations in only Flg increase the risk of inflammation and enhance S. aureus entry. Other epidermal barrier proteins, such as envoplakin, periplakin, and involucrin, may also control microbiome penetration into the skin (Natsuga et al., 2016). Barrier defects permitted increased S. aureus entry and subsequent enhanced expression of Th2 cytokines, IL-17, and thymic stromal lymphopoietin and decreased expression of cathelicidin. These results are consistent with previous reports showing that Th2 cytokines directly down-regulate the induction of cathelicidin in the skin (Howell et al., 2006b). Such changes are characteristic of AD and may illustrate how mutations of FLG in the human population may confer risk of AD by enabling the abnormal entry of microbes into the dermis.

We previously reported that most of the microbes in the dermis of human skin are not present within classical CD11c<sup>+</sup> phagocytic immune cells (Nakatsuji et al., 2013). Similarly, S. aureus was detected outside of CD11c<sup>+</sup> cells in lesional AD skin. These data suggest that these microbes entered across the epidermal barrier rather than being carried in by phagocytosis. Here, we directly showed in cultured skin equivalents that antigen-presenting cells are not required for S. aureus to enter the dermis. In contrast, penetration of the stratum corneum by S. aureus was dependent on protease activity. Future work will determine if a specific protease mediates entry, although there are several proteases produced by Staphylococcus species that are of interest. For example, S. aureus produces an extracellular zinc-calcium–dependent metalloproteinase called aureolysin, which can proteolytically degrade cathelicidin and neutralize its antimicrobial activity (Sieprawska-Lupa et al., 2004). S. aureus also produces a serine protease, commonly referred to as V8 protease, that is known to impair epidermal barrier function in mice (Hirasawa et al., 2010) and has been known as the “epidermolytic toxin” (Dancer et al., 1990; Redpath et al., 1991). S. aureus strains isolated from AD patients have been found to produce high extracellular proteolytic activity, and aureolysin and V8 protease predominantly contribute to their total proteolytic activity (Miedzobrodzki et al., 2002). In the current study we showed that the extracellular protease-null mutant of methicillin-resistant S. aureus USA300, which lacks both proteases, had less capacity to break through the epidermal barrier of the murine skin and organotypic human skin constructs. Thus, aureolysin and the V8 serine protease are two prime specific candidates to examine for their roles in permitting bacteria to penetrate the skin and may explain the higher amount of S. aureus seen in the dermis of AD subjects. Blocking specific bacterial proteases could therefore be a useful therapeutic approach for AD, although this therapeutic use of protease inhibitors for AD has been controversial (Foelster Holst et al., 2010; Wachter and Lezdey, 1992).

This study shows that S. aureus can directly penetrate the stratum corneum and epidermis, a behavior that explains how this microbe can disrupt skin immune homeostasis. Defects in the skin barrier enable enhanced entry from the surface, and although this entry does not show characteristics of infection, this penetration alters cytokine and AMP responses. Such inflammatory triggers may then further alter the surface microbiome and perpetuate disease by adding to the process of barrier disruption. Notably, we showed that barrier repair reduces S. aureus penetration into skin and normalizes immune abnormality triggered by the S. aureus penetration. Thus, these observations provide clinical insight into how physical and innate immune barrier defects influence the pathogenesis of AD and provide guidance for optimizing therapeutic approaches to this disorder.

MATERIALS AND METHODS
Analysis of skin from human subjects with AD
All sample acquisition, including biopsy samples of lesional and nonlesional skin from patients with AD and normal skin from non-AD subjects, was approved and performed in accordance with the Human Research Protections Program at the University of California–San Diego (reference number: 071032). Written informed consent was obtained from all subjects before performing
the skin biopsies. Demographic data of AD patients recruited are shown in Supplementary Table S1 online. Subepidermal compartments of lesional and nonlesional skin from patients with AD were excised from the skin biopsy sample by using LCM without inclusion of appendageal structures. Total genomic DNA was extracted from LCM sections and subjected to qPCR or pyrosequencing for 16S rRNA using universal 16S rRNA or species-specific primers/probe (see Supplementary Table S2 online). For details, see the Supplementary Materials online.

Tracking bacteria entry into mouse skin

All animal protocols were reviewed and approved by the University of California—San Diego (approval number: S09074). After shaving and disinfecting the dorsal skin of OVA-sensitized Flg\textsuperscript{LAC} or Camp\textsuperscript{-} mice, an agar disk (6 mm) containing S. aureus ATCC35556 or methicillin-resistant S. aureus USA300 LAC strains (1 \times 10^6 colony-forming units) was applied on the skin, and the entire dorsal skin was then covered with wound dressing film for 20 hours. An agar disk without bacteria or with UV-killed S. aureus (1 \times 10^7 colony-forming units equivalent) was used as control. After disk application, skin was carefully cleaned with alcohol swabs and frozen in tissue-embedding compound. Epidermis, dermis, and adipose tissue sections were excised by LCM without inclusion of appendageal structures. Total genomic DNA was extracted from each section for qPCR. For details, see the Supplementary Materials.

Skin barrier disruption and restoration

A sterile patch with OVA solution or phosphate buffer saline was placed on tape-stripped dorsal skin of Flg\textsuperscript{LAC} Balb/c mice for 8 days (the patch was replaced every 2 days) as described previously (Jin et al., 2009). Each mouse received three 8-days exposures in 2-week intervals. Twenty-four hours after the third sensitization, dorsal skin was tape-stripped. For skin barrier repair experiments an optimized formula of cholesterol, ceramide, linoleate, and palmitate (3:1:1:1) or propylene glycol-ethanol (7:3) (vehicle) was applied twice every 4 hours as previously described (Man et al., 1996; Mao-Qiang et al., 1995). S. aureus was applied 4 hours after the second application of barrier repair mixture or vehicle. For details, see the Supplementary Materials.

Data access

The 16S pyrosequencing data for this study have been submitted to DDBJ Sequence Read Achieve (http://trace.ddbj.nig.ac.jp/dra/index_e.html) through the Bioproject ID PRJDB4882 and published under the Accession Code DRA004759.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad, La Jolla, CA). Independent t test was used for significance of differences.

CONFLICT OF INTEREST

RLG is a scientific consultant for Sente Inc. and Matrisys Bioscience.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.05.127.

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