MICROBIOME

Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis

Teruaki Nakatsuji,[1] Tiffany H. Chen,[1] Saisindhu Narala,[1] Kimberly A. Chun,[1] Aimee M. Two,[1] Tong Yun,[1] Faiza Shafiq,[1] Paul F. Kotol,[1] Amina Bouslimani,[2] Alexey V. Melnik,[2] Haythem Latif,[3] Ji-Nu Kim,[3] Alexandre Lockhart,[4] Keli Artis,[4] Gloria David,[4] Patricia Taylor,[5] Joanne Streib,[5] Pieter C. Dorrestein,[2,6] Alex Grier,[7] Steven R. Gill,[7] Karsten Zengler,[3] Tissa R. Hata,[1] Donald Y. M. Leung,[5] Richard L. Gallo[1]*

The microbiome can promote or disrupt human health by influencing both adaptive and innate immune functions. We tested whether bacteria that normally reside on human skin participate in host defense by killing *Staphylococcus aureus*, a pathogen commonly found in patients with atopic dermatitis (AD) and an important factor that exacerbates this disease. High-throughput screening for antimicrobial activity against *S. aureus* was performed on isolates of coagulase-negative *Staphylococcus* (CoNS) collected from the skin of healthy and AD subjects. CoNS strains with antimicrobial activity were common on the normal population but rare on AD subjects. A low frequency of strains with antimicrobial activity correlated with colonization by *S. aureus*. The antimicrobial activity was identified as previously unknown antimicrobial peptides (AMPs) produced by CoNS species including *Staphylococcus epidermidis* and *Staphylococcus hominis*. These AMPs were strain-specific, highly potent, selectively killed *S. aureus*, and synergized with the human AMP LL-37. Application of these CoNS strains to mice confirmed their defense function in vivo relative to application of nonactive strains. Strikingly, reintroduction of antimicrobial CoNS strains to human subjects with AD decreased colonization by *S. aureus*. These findings show how commensal skin bacteria protect against pathogens and demonstrate how dysbiosis of the skin microbiome can lead to disease.

INTRODUCTION

Antimicrobial peptides (AMPs) are essential immune defense molecules produced by neutrophils, paneth cells, mast cells, epithelial cells, and adipocytes (*1*–*4*). Because of their direct antimicrobial action, AMPs control growth of microorganisms that normally reside on epithelial surfaces, a community of microbes referred to as the "microbiome" (*5*). The skin microbiome is also regulated by other ecological factors such as humidity, temperature, pH, and lipid content (*6*). As a consequence of the environment established by the host, only a restricted collection of microbes can normally colonize the epithelial surface. However, in some disease states, an altered balance of the microbiota can occur, a condition known as dysbiosis. This state of dysbiosis may contribute to the disruption of immune homeostasis and increase disease symptoms. For example, atopic dermatitis (AD) is a disease characterized by dry, itchy, and inflamed skin that is frequently subject to infections of the skin and has been clearly shown to have an altered skin bacterial flora when compared to non-AD subjects (*7*, *8*). Although it is not clear why this occurs, the skin of subjects with AD has a decreased capacity to express certain AMPs such as cathelicidins and β-defensins despite the presence of skin inflammation (*9*–*13*). This relative deficiency in AMPs has been proposed to result in an inability of the skin to resist growth of pathogens such as *Staphylococcus aureus*.

The high abundance of *S. aureus* in AD contributes to the pathophysiology of this disease and has been linked with immune dysfunction including T helper cell 2 lymphocyte skewing (*14*), reduced AMPs (*12*, *15*), exacerbated allergic reactions (*16*, *17*), and disruption of the skin barrier (*18*). Mice develop AD-like lesions when they are experimentally colonized by *S. aureus* (*19*, *20*). However, in contrast to the exacerbation of disease and inflammation caused by *S. aureus*, other bacterial species found on normal skin appear to aid in normal immune homeostasis (*21*). For example, *Staphylococcus epidermidis*, a prevalent species of bacteria on healthy human skin, can diminish inflammation after injury (*22*), enhance development of cutaneous T cells (*23*–*25*), and promote expression of AMPs (*23*, *24*, *26*–*28*). Furthermore, some bacteria have been shown to produce their own AMPs that can synergize with AMPs produced by mammalian cells (*29*, *30*).

Here, we hypothesized that normal human skin is populated by commensal bacteria that promote health by producing antimicrobial activity. Furthermore, we asked whether a deficiency of this community in AD might exacerbate disease by facilitating the survival of *S. aureus*. We report a discovery of a broad community of protective bacteria on normal human skin that provides host defense against pathogens. These observations offer a specific strategy for rational microbiome therapy.

RESULTS

Bacteria on normal human skin inhibit *S. aureus* but are infrequent in AD

We performed 16*S* rRNA gene sequencing and culture-based analysis of skin swabs taken from healthy non-AD subjects and the lesional and

[1]Department of Dermatology, University of California, San Diego, La Jolla, CA 92092, USA. [2]Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92092, USA. [3]Department of Bioengineering, University of California, San Diego, La Jolla, CA 92092, USA. [4]Rho Federal Systems Division Inc., Chapel Hill, NC 27517, USA. [5]Division of Allergy and Immunology, Department of Pediatrics, National Jewish Health, Denver, CO 80206, USA. [6]Departments of Chemistry, Biochemistry and Pharmacology, University of California, San Diego, La Jolla, CA 92092, USA. [7]Department of Microbiology and Immunology, University of Rochester, Rochester, NY 14611, USA.
*Corresponding author. Email: rgallo@ucsd.edu

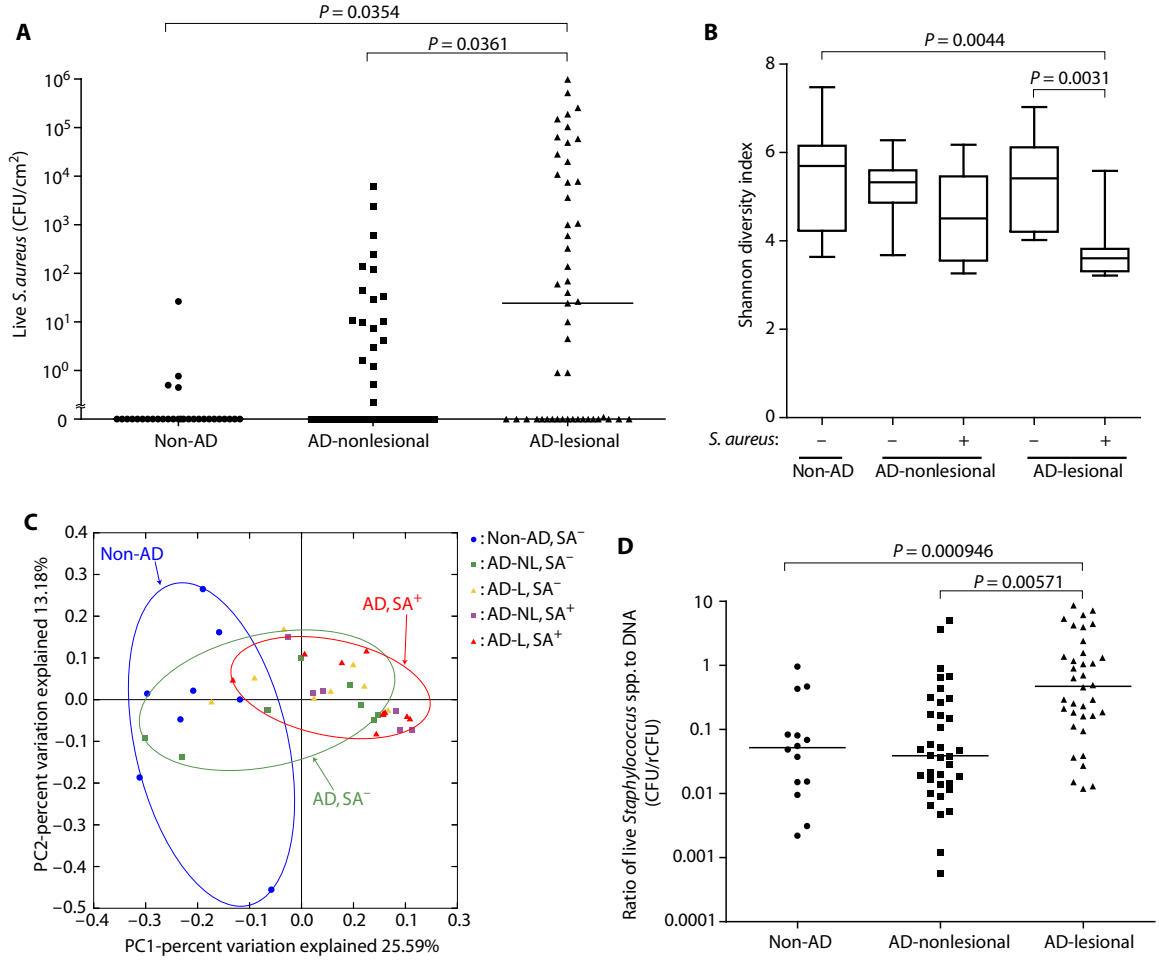


Fig. 1. Dysbiosis of the skin microbiome in AD is associated with *S. aureus* colonization. (**A**) Live *S. aureus* CFUs on skin of non-AD subjects and on nonlesional and lesional skin of subjects with AD. Bar, median. (**B**) Shannon diversity index of bacterial community on skin from non-AD and *S. aureus* culture–positive or *S. aureus* culture–negative subjects with AD. Data are shown as box and whisker plots. (**C**) Principal coordinate analysis plot analysis displaying composition of bacterial communities on non-AD and *S. aureus* culture–positive or *S. aureus* culture–negative subjects with AD. SA^+, *S. aureus* culture–positive; SA^-, *S. aureus* culture–negative; L, lesional; NL, nonlesional. (**D**) Ratio of *Staphylococcus* spp. CFU abundance determined by live colony counting compared to rCFU of *Staphylococcus* determined by qPCR with species-specific primers. Bar, median. *P* values were calculated by two-tailed paired *t* test for lesional versus nonlesional samples or two-tailed independent *t* test for non-AD versus AD groups.

nonlesional sites of subjects with AD (refer to table S1 for demographic data). A large proportion of subjects with AD were identified to be culture-positive for *S. aureus* on both their lesional and nonlesional skin (Fig. 1A). *S. aureus* culture–positive lesional skin had decreased bacterial diversity when compared to lesional skin that was *S. aureus* culture–negative (Fig. 1B and fig. S1). Principal coordinate analysis using weighted UniFrac distances suggested a similarity in the bacterial communities of non-AD controls and *S. aureus*–positive and *S. aureus*–negative AD subjects (Fig. 1C). The absolute abundance of total staphylococcal species on skin swabs was also evaluated by manual colony counting for colony-forming units (CFUs) on a selective egg yolk mannitol salt agar (fig. S2A) and by quantitative polymerase chain reaction (qPCR) of bacterial DNA with genus-specific 16S primers to yield a relative CFU (rCFU) based on DNA abundance (fig. S2B). Swabs to quantify live bacteria and bacterial DNA were simultaneously obtained from the area next to each other. The specificity of the *Staphylococcus*-specific primers was confirmed as shown in fig. S3. Both colony counting and 16S rDNA qPCR methods gave similar results from samples from AD lesional skin. However, these methods yielded different results on samples from normal skin where qPCR of DNA abundance detected about 10-fold higher rCFUs than live colony counting (Fig. 1D). One explanation for the larger amount of DNA recovered from normal skin compared to AD skin was that DNA recovered from normal skin was associated with bacteria that had been killed by the normal antimicrobial defense systems on the skin surface environment. This observation led us to hypothesize that bacteria residing on the skin of AD subjects had less antimicrobial activity than those on normal skin.

Previous studies have found that laboratory isolates of coagulase-negative *Staphylococcus* (CoNS) species can produce AMPs (*29*, *30*). We therefore next sought to determine the frequency of CoNS strains that produce antimicrobial activity on normal skin. To detect antimicrobial activity, we randomly isolated about 80 individual CoNS isolates from each skin culture swab and then conducted an unbiased analysis of antimicrobial activity by measuring the capacity of the sterile conditioned supernatant of each individual isolate to inhibit *S. aureus* growth (Fig. 2A). A total of 2029 colonies were studied from the forearms of 30 healthy subjects, and 5695 colonies were studied from

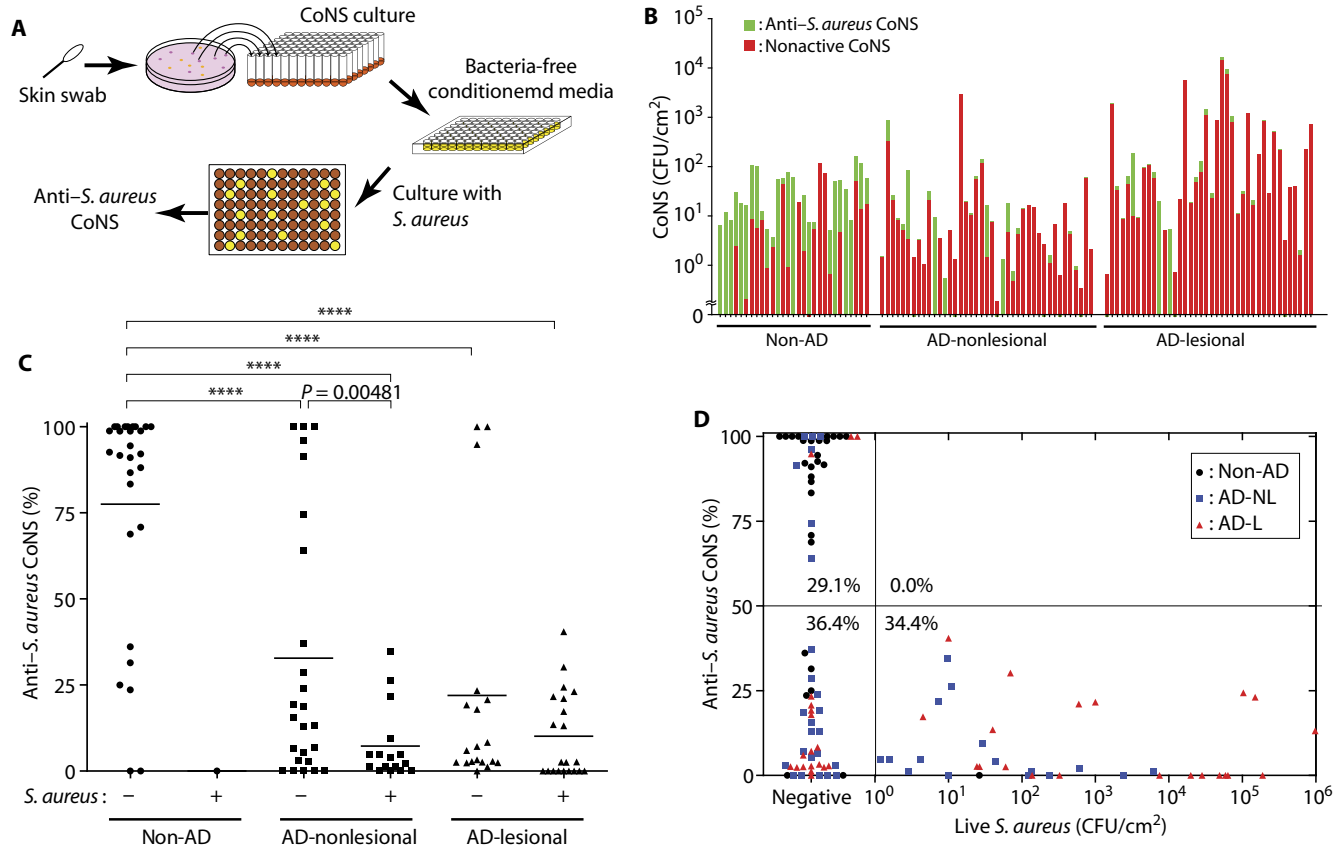


Fig. 2. *S. aureus* colonization correlates with a lack of antimicrobial activity in CoNS. (**A**) Schematic of a high-throughput antimicrobial screening of CoNS against *S. aureus*. (**B**) CFU of CoNS without (red) or with antimicrobial activity against *S. aureus* (green). Each bar represents data from individual subjects. (**C**) Frequency of CoNS colonies with activity against *S. aureus*. Data are reported for 29 healthy subjects and 41 nonlesional or 40 lesional sites from AD subjects. Subjects that had less than 25 CoNS colonies per swab were not included. Each point represents the frequency that anti–*S. aureus* activity was detected in all colonies isolated from each individual. Bar, mean. ****$P < 0.0001$. *P* values were calculated by two-tailed independent *t* test. (**D**) Correlation between proportion of CoNS strains that have the capacity to inhibit *S. aureus* growth and abundance of live *S. aureus* CFU on the skin surface. Each dot represents data from one individual. Quadrants are divided on the basis of the frequency of antimicrobial CoNS (>50 or <50%) and detection of live *S. aureus* (<1 or >1 CFU/ cm^2). The proportion (%) of subjects in each quadrant to total subjects is shown. L, lesional; NL, nonlesional.

nonlesional and lesional skin of 49 subjects with AD. This functional screening revealed that most CoNS clones isolated from healthy subjects could inhibit *S. aureus* growth, but CoNS clones with antimicrobial activity were much less frequent on AD subjects and were lowest on individuals who were *S. aureus* culture–positive (Fig. 2, B and C). Low frequency of CoNS with antimicrobial activity was also seen in the only healthy subject that was *S. aureus* culture–positive. The measurement of frequency of clones with anti–*S. aureus* activity was stable in multiple skin swab samples from the same subjects over 14 days (fig. S4). No subject that was culture-positive for *S. aureus* had antimicrobial activity detected in more than 50% of their CoNS isolates (Fig. 2D). This suggested that a deficiency in the antimicrobial function of the CoNS bacterial community was strongly associated with colonization by *S. aureus*.

To identify the bacteria that had antimicrobial activity, full-length 16*S* rRNA gene sequencing was performed on active clones (refer to table S2 for the number of clones analyzed). The predominant species of CoNS with anti–*S. aureus* activity was either *S. epidermidis* or *Staphylococcus hominis*, although other species were also observed with this activity (Fig. 3A and table S2, A and B). However, many clones of these CoNS species did not produce antimicrobial activity, thus demonstrating that the strain, and not only species identity, predicts antimicrobial function (Fig. 3B and table S2B).

Application of antimicrobial CoNS strains to skin inhibits *S. aureus*

To confirm that the antimicrobial function detected through random screening in vitro could be reproduced under the physiologically relevant conditions of the skin, the *S. hominis* clone A9 was selected for further analysis. This strain was selected because *S. hominis* was frequently detected in our screen, and the function of *S. hominis* on human skin was unknown. *S. hominis* A9 grown on agar generated a clear zone of inhibition of *S. aureus* growth, thus confirming that this strain spontaneously secreted activity into culture medium at a level sufficient to directly inhibit *S. aureus* (Fig. 4A). To directly test whether this clone could reduce colonization by *S. aureus* on the skin, we applied *S. hominis* A9 or an equal density of control *S. hominis* isolates without antimicrobial activity to the surface of sanitized pigskin (*31*) on which a defined amount of *S. aureus* had first been applied. The amount of *S. hominis* applied was similar to the estimated density of bacteria on normal human skin (1×10^5 CFU/cm^2). A significant decrease in *S. aureus* was seen after a single application of *S. hominis* A9 at this density ($P = 0.0276$; Fig. 4B). Application of *S. hominis* A9 that was killed before application or application of control *S. hominis* strains that did not show antimicrobial activity did not reduce *S. aureus* survival. Similarly, after colonization of the back skin of mice with

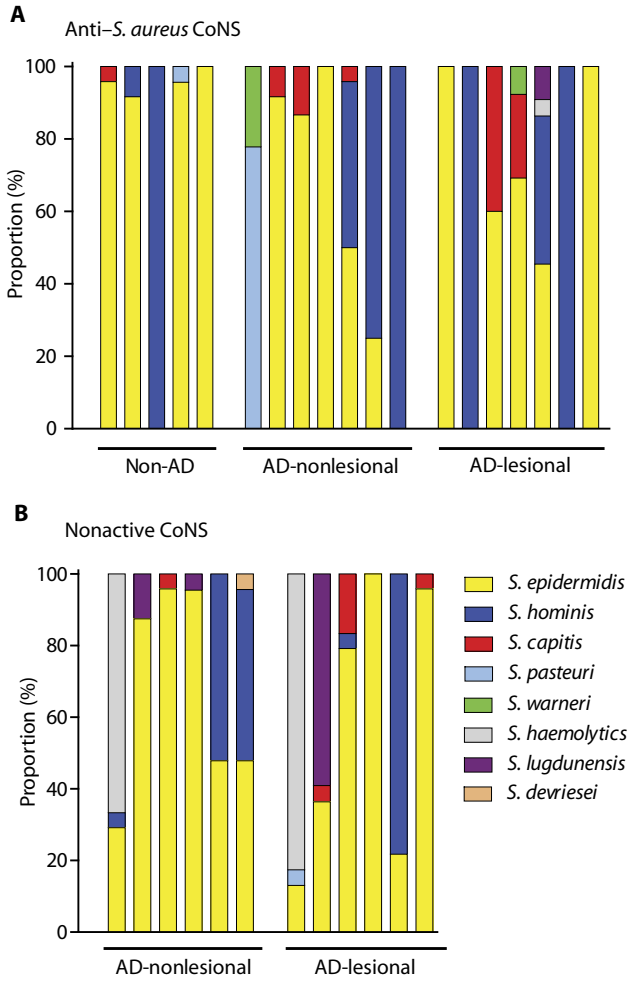


Fig. 3. Antimicrobial activity is detected in diverse strains of CoNS and not predictable at the species level. (**A**) Relative abundance of CoNS species with antimicrobial activity from five non-AD subjects and seven subjects with AD, who were randomly selected. Up to 48 CoNS isolates were randomly selected from each individual for 16*S* rRNA sequencing to identify species. Refer to table S2 for details. (**B**) Relative abundance of CoNS species without anti–*S. aureus* activity isolated from six subjects with AD. Refer to table S2 for details.

a known amount of *S. aureus*, a single application of *S. hominis* A9 reduced *S. aureus* but control strains of *S. hominis* had no effect (Fig. 4C). Furthermore, twice-a-day applications of *S. hominis* A9 for 1 week completely eliminated *S. aureus* colonization compared to vehicle (Fig. 4D). Vehicle treatment did not affect *S. aureus* survival on the skin surface in comparison to untreated control (fig. S5). These observations show that *S. hominis* A9 produced anti–*S. aureus* activity when present on the skin and prompted further biochemical and genetic identification of the molecule(s) responsible for this activity.

Commensal bacteria on human skin produce AMPs

Reverse-phase chromatography revealed two independent peaks with antimicrobial activity in the conditioned medium of *S. hominis* A9 (Fig. 5A and fig. S6). Edman degradation, whole-genome sequencing, and genome-guided matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF)/TOF analyses predicted the structures for the mature forms of these AMPs as lantibiotics (Fig. 5B and fig. S7). These proteins were encoded within a gene cluster that included *lanM*, *lanC*, and *lanT* homologs (Fig. 5, C and D), and were duplicated within the genome, but not detected within the genome of another *S. hominis* strain previously sequenced (*32*). Because these *S. hominis* AMPs had similar structure to known lantibiotics (*33*), we refer to them henceforth as *Sh*-lantibiotic-α and *Sh*-lantibiotic-β. To validate the antimicrobial function of *Sh*-lantibiotic-α and *Sh*-lantibiotic-β on skin, we applied 0.5 nmol of purified *Sh*-lantibiotics to sanitized pigskin to which *S. aureus* had been previously applied. Direct application of these *Sh*-lantibiotic peptides to skin, or application of sterile conditioned culture medium from *S. hominis* A9, significantly inhibited *S. aureus* ($P < 0.01$; Fig. 5E). In contrast, these *Sh*-lantibiotics did not act against *S. hominis* A9 itself at concentrations up to 10 μM (Fig. 5F). *S. hominis* A9 strongly suppressed growth of multiple strains of *S. aureus*, including MRSA strain USA 300, and clinical isolates from subjects with AD but did not affect growth of other species frequently isolated from normal human skin, including *Propionibacterium acnes*, *S. epidermidis*, and *Corynebacterium minutissimum* (fig. S8).

Identification of the genes encoding *Sh*-lantibiotics permitted us to screen subjects by direct colony PCR to determine the frequency of this AMP in the CoNS community residing on the skin of human subjects. PCR analysis was performed on 632 CoNS colonies from the skin of 9 randomly selected normal subjects and 1713 CoNS colonies from 11 randomly selected individuals with AD. These results showed that the *Sh*-lantibiotic-α genes were common in the bacterial community on normal subjects but not on AD subjects (Fig. 6A), a finding consistent with our functional screening results shown earlier in Fig. 2. Western blot analysis using mouse polyclonal immunoglobulin G raised against *Sh*-lantibiotic-α peptide showed that the peptide could be detected on the skin of non-AD subjects that were PCR-positive for the *Sh*-lantibiotic-α gene, but not on AD subjects that were PCR-negative (Fig. 6B and fig. S9), thus confirming that the protein for this lantibiotic was constitutively expressed on human skin and correlated with the presence of the gene. These *Sh*-lantibiotics were likely not the only antimicrobials produced by the skin microbiome because genome sequencing of additional clones selected by functional screening revealed other putative lantibiotic and bacteriocin genes in other active isolates (fig. S10). We also identified previously known lantibiotics, such as epidermin and Pep-5 (*34*), in the genomes of some *S. epidermidis* clones with anti–*S. aureus* activity isolated from normal skin (fig. S11). Lantibiotic-related genes were not found in the genomes of strains that did not exhibit antimicrobial activity such as *S. hominis* C4, C5, and C6.

Sh-lantibiotics synergize with a human AMP

The human skin expresses several human AMPs, and therefore, these host AMPs will be present together with the bacterial *Sh*-lantibiotics on the skin surface. This shared location raised the potential that the bacterial AMPs may act with human AMPs to defend against pathogens. To examine this, we combined each *Sh*-lantibiotic with LL-37, a mammalian AMP of the cathelicidin family. *Sh*-lantibiotic-α and *Sh*-lantibiotic-β showed strong activity alone and had superior activity against *S. aureus* compared to known human AMPs (Fig. 6, C and D) (*35*). LL-37 alone (8 μM) decreased *S. aureus* survival by >3-log, whereas 4 μM LL-37 alone did not affect *S. aureus* survival in vitro. Combination with 4 and 8 μM LL-37, concentrations consistent with those measured on human skin, enhanced the antimicrobial activity of each *Sh*-lantibiotic by 4-fold (with 4 μM LL-37) and 16-fold (*Sh*-lantibiotic-α with 8 μM LL-37) or 32-fold (*Sh*-lantibiotic-β with 8 μM LL-37), respectively, suggesting synergistic action between prokaryotic and host AMPs.

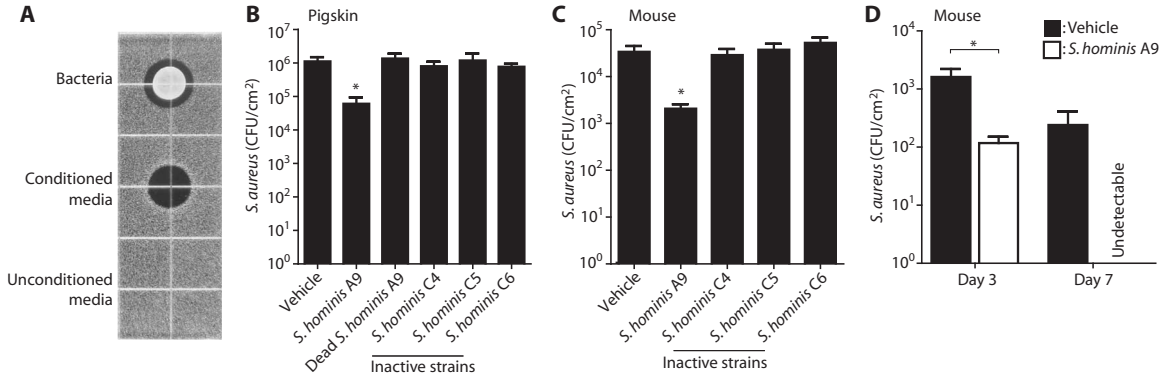
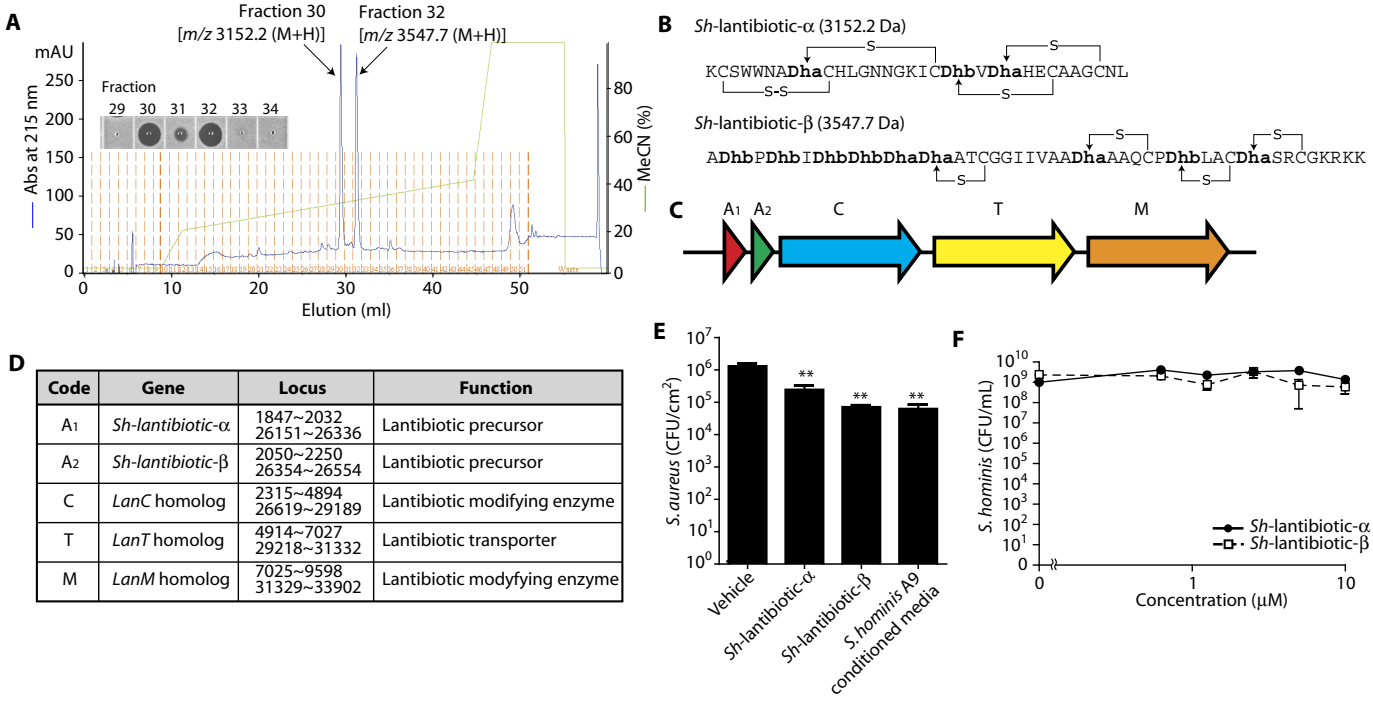


Fig. 4. Colonization by an antimicrobial CoNS strain is protective against *S. aureus*. (**A**) Anti–*S. aureus* activity secreted from a representative antimicrobial *S. hominis* strain (A9) isolated from a non-AD subject. A live colony of *S. hominis* A9 (upper), conditioned medium (10 μl) from an *S. hominis* A9 overnight culture (middle), or unconditioned medium [tryptic soy broth (TSB)] served as a negative control were applied on TSB agar containing *S. aureus*. The black area represents zone of growth inhibition of *S. aureus*. (**B** and **C**) Effect of *S. hominis* on the survival of *S. aureus* on ex situ pigskin (B) or *S. hominis* applied to live mouse skin (C). *S. aureus* was first applied to skin as described in Materials and Methods. The action of *S. hominis* A9 was compared to controls, including UV-killed and washed A9 or live *S. hominis* strains that do not produce AMP activity in solution assay (C4, C5, and C6). All CoNS bacteria were applied at 1×10^5CFU/cm^2. Data represent means ± SEM of data from five pigskin sheets (B) or six mice (C). (**D**) Effect of multiple applications of *S. hominis* A9 on survival of *S. aureus* on mouse skin. *S. hominis* A9 strain or vehicle was applied twice a day to mouse back skin colonized by *S. aureus* over the indicated periods. Skin swabs were collected to count *S. aureus* survival. Data represent means ± SEM from 10 (day 3) or 5 (day 7) independent mice. *$P < 0.05$ by two-tailed independent *t* test.



Code	Gene	Locus	Function
A_1	*Sh-lantibiotic-α*	1847~2032 26151~26336	Lantibiotic precursor
A_2	*Sh-lantibiotic-β*	2050~2250 26354~26554	Lantibiotic precursor
C	*LanC* homolog	2315~4894 26619~29189	Lantibiotic modifying enzyme
T	*LanT* homolog	4914~7027 29218~31332	Lantibiotic transporter
M	*LanM* homolog	7025~9598 31329~33902	Lantibiotic modyfying enzyme

Fig. 5. *S. hominis* A9 isolated from normal human skin produces unique lantibiotics. (**A**) Reverse-phase high-performance liquid chromatography elution profile of peptides purified from culture supernatant of *S. hominis* A9 strain. The insert is a radial diffusion activity against *S. aureus* from the indicated fractions. Molecular mass of fractions 30 and 32 was measured by MALDI-TOF mass spectrometry (MS) (fig. S4). (**B**) Amino acid sequence and predicted thioester bonds from the two lantibiotics purified from *S. hominis* A9. Dha, 2,3-didehydroalanine; Dhb, (*Z*)-2,3-didehydrobutyrine. (**C**) Organization of the gene cluster encoding *Sh*-lantibiotic precursors and lantibiotic biosynthetic genes in *S. hominis* A9. (**D**) List of lantibiotic-related genes, gene locus, and putative functions. (**E**) Effect of application of *Sh*-lantibiotics (0.5 nmol) or conditioned medium from *S. hominis* A9 (50 μl) on survival of *S. aureus* on pigskin. Data represent means ± SEM of four independent assays. **$P < 0.01$ by two-tailed independent *t* test. (**F**) Dose-response curves for the antimicrobial activity of *Sh*-lantibiotic-α and *Sh*-lantibiotic-β against *S. hominis* A9 strain. Data represent means ± SEM of triplicate assays. *m/z*, mass/charge ratio.

Application of bacteria with antimicrobial activity decreased *S. aureus* in AD

To investigate the capacity of commensal bacteria to inhibit *S. aureus* on human skin, we tested the effect of applying these bacteria to subjects with AD. Five AD subjects who were *S. aureus* culture–positive were recruited to participate in this study (table S3). Strains with antimicrobial activity were infrequent within the total CoNS community on these AD subjects, but rare clones could be isolated if sufficient

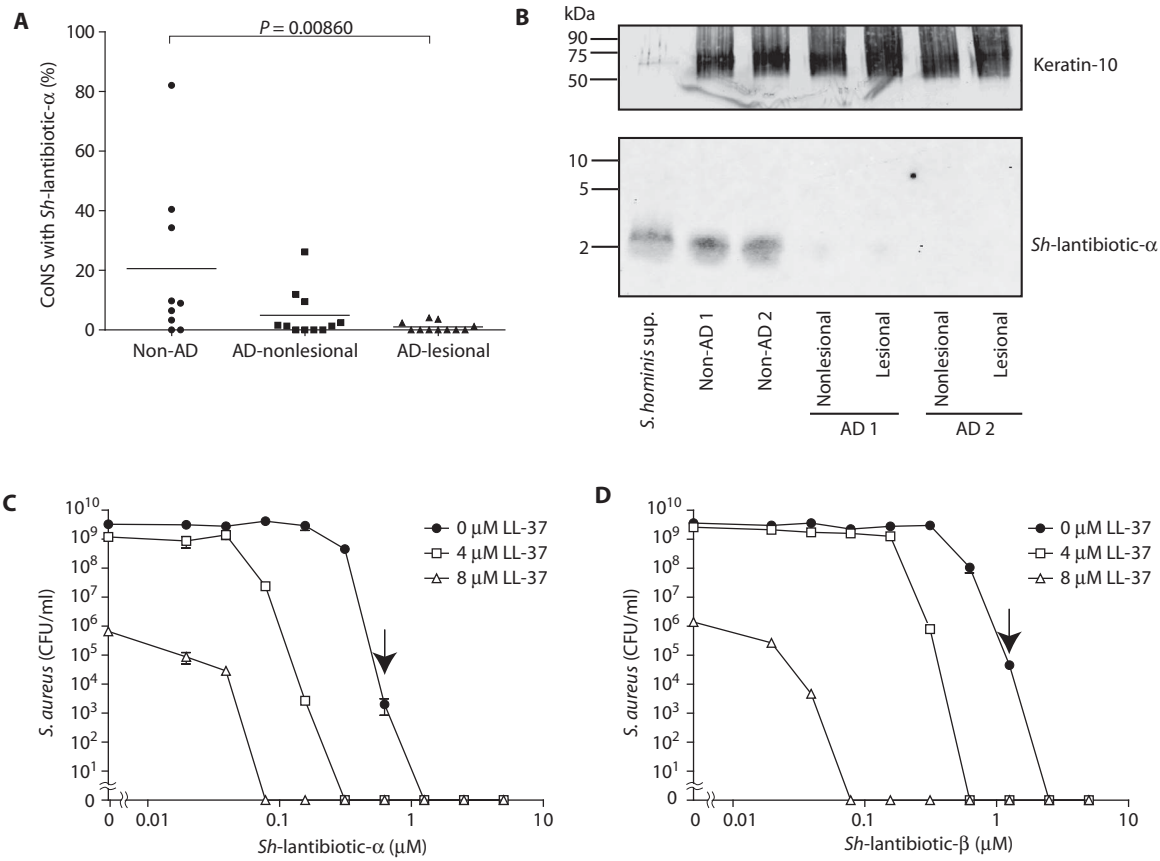


Fig. 6. *Sh*-lantibiotics are commonly found on healthy human skin and synergize with a host AMP. (**A**) Frequency of detecting *Sh*-lantibiotic-α by colony PCR using gene-specific primers in CoNS isolates from human skin. Each point represents analysis of one individual. Bar, mean. *P* value was calculated by Wilcoxon-Mann-Whitney test. (**B**) Detection of *Sh*-lantibiotic-α peptide by Western blotting from extracts of skin swabs taken from two non-AD subjects who were colonized by bacteria having the *Sh*-lantibiotic-α gene and two AD subjects who were PCR-negative for the *Sh*-lantibiotic-α gene. *S. hominis* culture supernatant was loaded as a positive control. A total of 20 μg of protein was loaded in each lane. The uncropped image is shown in fig. S9. The membrane was restained with antibody against cytokeratin-10, a predominant protein in the stratum corneum, as a loading control. (**C** and **D**) Dose-response curves for the antimicrobial activity of *Sh*-lantibiotic-α (C) and *Sh*-lantibiotic-β (D) against *S. aureus* and their synergistic antimicrobial activity with human LL-37. Data represent means ± SEM of triplicate assays. Arrow shows minimal bactericidal concentration.

CoNS colonies were screened. Only a single *S. epidermidis* or *S. hominis* strain with antimicrobial activity was isolated from the three subjects. In these cases, a single strain of CoNS was formulated (refer to Fig. 7A). Three and two antimicrobial *S. hominis* or *S. epidermidis* strains were isolated from two AD subjects. The whole genome of each active clone was sequenced, and clusters of lantibiotic- or bacteriocin-related genes were identified in all active clones (Fig. 7A and fig. S10). All selected clones and their conditioned media were confirmed to produce a zone of inhibition of *S. aureus* growth by radial diffusion assay (Fig. 7B). These clones with antimicrobial activity were then expanded and formulated in a cream vehicle base (Cetaphil lotion), and a single dose was applied in a double-blind fashion to the forearm of each subject, whereas vehicle alone was applied to the contralateral arm (autologous transplant) (refer to fig. S12 for study design). Bacteria were applied to the skin for a final concentration of 1×10^5 CFU/cm^2, a density similar to previous assessments of the abundance of bacteria on normal human skin. Subjects were instructed not to wash for 24 hours after application. *S. aureus* was measured at each site before and 24 hours after application of active CoNS transplant or vehicle. To assess potential vehicle effects, we left some subjects untreated. Twenty-four hours after initiation of the study, no significant difference in *S. aureus* abundance was detected between untreated individuals and individuals treated with vehicle alone (Fig. 7C), although in the absence of washing, a trend toward an increase in bacterial abundance from baseline was seen in both untreated and vehicle-treated groups (fig. S13). In contrast, a single application of antimicrobial CoNS strain(s) significantly decreased *S. aureus* abundance compared to vehicle (P = 0.0402; Fig. 7C and fig. S13). These data confirmed that CoNS strains with antimicrobial activity can directly suppress *S. aureus* colonization on human subjects with AD.

DISCUSSION

AD is a common and severe skin disorder that harbors an abnormal community of bacteria on the skin. In particular, patients with AD are frequently colonized by *S. aureus* that then exacerbates the disease by further promoting inflammation (*7*, *8*). We hypothesized that the commensal bacteria residing on normal skin might contribute to host defense by producing molecules that inhibit colonization by *S. aureus*. Furthermore, we examined whether the dysbiosis seen in AD may be functionally important because of a loss of these protective strains. We observed that several different bacterial species on healthy human skin produce anti–*S. aureus* activity and that bacteria with this activity were far less frequent on AD subjects. The relative

A

Subject ID	% Anti–*S. aureus* CoNS	CoNS clones	Species	Antimicrobial class
AMT1	1.2	AMT1-A9	*S. epidermidis*	Lantibiotic
AMT2	0.6	AMT2-A12	*S. hominis*	Lantibiotic
AMT3	1.2	AMT3-A12	*S. hominis*	Lantibiotic
AMT4	15.5	AMT4-C2	*S. hominis*	Lantibiotic
		AMT4-D12	*S. hominis*	Lantibiotic
		AMT4-G1	*S. hominis*	Bacteriocin
AMT5	1.8	AMT5-C5	*S. epidermidis*	Bacteriocin
		AMT5-G6	*S. epidermidis*	Bacteriocin

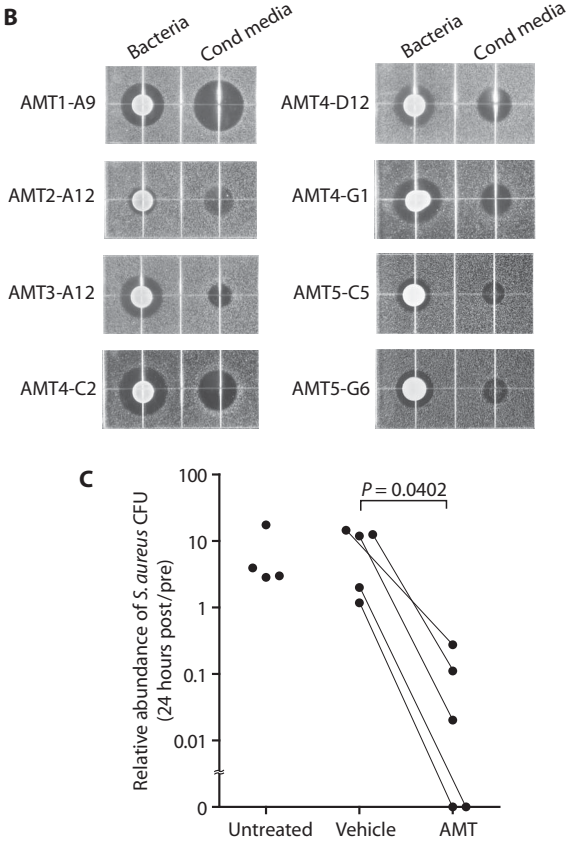


Fig. 7. Transplantation of antimicrobial CoNS reduces survival of *S. aureus* on human skin. (**A**) Characterization of CoNS clones used for autologous microbiome transplant (AMT). Antimicrobial class of each clone was identified by whole-genome sequencing (refer to fig. S11 for more details). (**B**) Radial diffusion assay for anti–*S. aureus* activity secreted from each active CoNS strain used for AMT. Radial diffusion assay of bacteria and conditioned medium was conducted as described previously. (**C**) Effect of transplantation of antimicrobial CoNS or vehicle on the survival of *S. aureus* on the skin of five subjects with AD. *S. aureus* survival was measured by colony counting before transplant (pre) and 24 hours after a single application of bacteria (post). Application and analysis were done in a blinded fashion, and samples are from the contralateral arm of subjects treated with the identical vehicle containing antimicrobial bacteria or vehicle only. Data of AMT or placebo treatment (vehicle) are compared with data from four untreated control subjects with AD. *P* value was calculated by two-tailed paired *t* test.

absence of commensal bacterial strains producing antimicrobial activity was most evident on individuals colonized by *S. aureus*. Indeed, no subject was colonized by *S. aureus* if they also had a normal abundance of CoNS bacteria that produced antimicrobial activity, and application of antimicrobial CoNS strains to animal or human skin greatly reduced *S. aureus* colonization. Together, these observations show that specific bacteria within the human skin microbiome defend against *S. aureus*.

In normal skin, culture-based methods for estimating CFU detected a lower abundance of bacteria than DNA-based methods. This was not likely to be inherent to the techniques themselves because the two methods yielded similar results when samples were taken from AD lesional skin. There are several potential explanations for this observation, including differences in capacity to sample bacterial DNA on AD lesional skin compared to healthy skin, or other products in the samples influencing the assay results. However, it is also possible that more bacteria on normal skin are dead than on AD lesional skin. Indeed, we have shown here that normal skin has more effective surface antimicrobial activity than AD skin. AMPs such as LL-37, β-defensin-2, and β-defensin-3 also have lesser expression in inflamed skin of AD patients than in inflamed skin of normal subjects, but the constitutive expression of these AMPs is low in noninflamed skin (*10*–*13*, *36*). Therefore, the increased capacity of noninflamed normal skin to kill bacteria is not likely due to the expression of these host AMPs. Alternatively, the higher antimicrobial activity generated by the commensal CoNS community on normal skin can explain this increased surface antimicrobial action. In this model, the microbiome provides the first line of defense, whereas the host innate immune system provides the second line of defense, which is activated only after the surface is damaged to trigger host AMP production.

Unbiased functional selection of commensal CoNS strains that were prevalent on human skin permitted identification of previously unknown AMPs. We report purification and validation of two potent lantibiotics in a common commensal strain of *S. hominis*. These lantibiotics were constitutively secreted by bacteria at concentrations that are sufficient to kill *S. aureus* on the skin surface, detectable on the surface of normal human skin, and highly synergistic with LL-37, an important human AMP (*2*, *37*). The synergistic action between prokaryotic and host AMPs suggests that the microbe-derived molecules should be considered as a component of the normal skin immune defense system. Several other bacterial species were also found to produce antimicrobial activity. Although Gram-negative bacteria have been rarely detected on normal human skin, it has been reported recently that selective culture systems could successfully expand some Gram-negative bacteria from healthy donors and that these could suppress *S. aureus* growth on AD subjects (*38*). It is unclear whether the presence of such Gram-negative bacteria influences normal host defense. Furthermore, other potential activities detected in laboratory strains of bacteria such as the capacity to inhibit biofilm formation (*39*) may also play a role in normal skin protection. A limitation of this current study is that a complete catalog of protective bacteria cannot be identified at this time. A wide range of microbes may have this function, and factors that influence their survival are not currently understood. Overall, these data suggest that the human skin microbiome could provide a diverse array of beneficial factors that may require functional screening to first detect.

The *Sh*-lantibiotics identified here showed selective activity against *S. aureus* but did not inhibit the growth of *S. hominis* and *S. epidermidis*. Other isolates of CoNS also exerted selective antimicrobial activity against *S. aureus* but not against bacterial species frequently found on the healthy skin. Previously identified AMPs from *S. epidermidis* were also known to exert selective killing, a logical behavior if the cell is to resist killing itself (*30*). The mechanism responsible

for selective killing by bacterial AMPs may be due to differences in the capacity to disrupt the microbial cell membrane. The capacity for selective killing of pathogenic bacteria over the normal microflora is highly desirable because it will help to shape the normal bacterial community. This illustrates the potential in further analysis of the host defense function of the human skin microbiome and is consistent with observations that expression of a bacteriocin in the intestinal microbiome can outcompete pathogens at the epithelial interface (*40*). Because we found that antimicrobial function of skin bacteria is not predictable at the species level, more detailed metagenomic (*41*), transcriptomic (*42*), proteomic, and metabolomic analyses (*43*, *44*) will provide a more complete understanding of the diverse antibacterial defense system of the skin.

We have shown here in both animal and human systems that topical application of rationally selected bacterial strains can decrease *S. aureus* abundance. This approach is inherently superior to current pharmaceutically derived antibiotics because they should not disrupt cutaneous homeostasis by nonspecific killing of the normal microflora. On the basis of our observations, nonspecific antibacterial action by common pharmaceutical antibiotics could kill protective strains of CoNS and might therefore enhance the potential for recolonization by *S. aureus*. The selective activity of antimicrobials derived from normal flora would avoid this nonspecific effect and illustrates why bacteriotherapy that kills *S. aureus* but does not inhibit other beneficial CoNS strains could be superior. Thus, application of a single selective CoNS AMP may permit emergence of multiple different AMPs, an environment less likely to promote antibiotic resistance to any single antibiotic. Long-term protection could be achieved if the applied antimicrobial bacteria could successfully colonize on the skin surface. We demonstrated that repeated application of active CoNS for 1 week completely eliminated *S. aureus* on mice. The benefits of long-term/multiple applications of antimicrobial bacteria on the skin of subjects with AD remain to be determined.

Enhancing the presence of certain CoNS strains could benefit the patient by also exerting anti-inflammatory actions. For example, *S. epidermidis* has been shown to be anti-inflammatory (*22*, *25*). Given the complex nature of AD, ideal bacteriotherapy would include both targeting repair of the intrinsic epidermal barrier and optimizing the immune defense functions provided by the resident bacteria. Some members of normal skin flora may be able to do both (*22*–*26*, *29*, *30*). Extended periods of bacteriotherapy will be necessary to evaluate this effect.

In conclusion, we report here evidence that the community of bacteria residing on normal human skin provides protection against *S. aureus*. A lack of this activity on the skin of subjects with AD is strongly associated with increased colonization by *S. aureus*. It is not clear at present whether this dysfunction of the bacterial community in AD was an intrinsic result of the disease itself or whether it is attributed to extrinsic factors in subjects with AD such as environmental exposure or the action of other members of the AD bacterial community to suppress commensal microbes that are beneficial and in turn resist *S. aureus*. The presence of *S. aureus* may also be contributing to the suppression of the protective strains of CoNS identified here. However, with the identification of unique antimicrobials and bacterial strains from healthy human skin, it is possible to exploit this information in diagnostically and therapeutically useful ways. Such observations will further define the important mutualistic relationship that exists between humans and some bacteria at the epithelial interface.

MATERIALS AND METHODS

Study design

This study was designed to test the hypothesis that the skin commensal bacteria contribute to the host defense by producing molecules that can kill *S. aureus*. Adults with AD and age-matched non-AD subjects were recruited from the University of California, San Diego (UCSD), La Jolla, CA, and the National Jewish Health (NJH), Denver, CO (demographic data are shown in table S1). All subjects avoided any treatments and therapies that may potentially affect skin microbiome before sample collection (refer to the Supplementary Materials for sampling collection criteria). Collection of surface bacteria was done from lesional skin on the antecubital fossa and from nonlesional skin of the upper arm. Microbial communities on the skin surface of healthy subjects and patients with AD were evaluated by culture-based colony counting, qPCR, and 16*S* rRNA community sequencing. Antimicrobial activity of CoNS isolated from the skin of healthy subjects and patients with AD was measured against *S. aureus* by high-throughput screening. The species of CoNS with or without anti–*S. aureus* activity were identified by 16*S* rRNA sequence. Antimicrobial activity produced by representative CoNS strain isolated from a non-AD healthy subject was characterized by liquid chromatography, MALDI-TOF/TOF MS, Edman end-terminal sequencing, and genome sequencing. To test the therapeutic potential of CoNS strains with anti–*S. aureus* activity, active strains were applied at physiologically relevant density on ex situ pigskin or mouse skin colonized by *S. aureus*. To further investigate the antimicrobial activity of CoNS in human, CoNS strains with anti–*S. aureus* activity were autologously reapplied to the lesional skin of one arm of the AD subject, whereas vehicle only was applied to the contralateral arm. All treatment was conducted in a double-blinded fashion and unblinded after all results were analyzed. No power analysis was performed to predetermine sample size. The number of experimental replications is provided in the figure legends. The details of all protocols and all primary data for experiments where $n < 20$ (table S5) are provided in the Supplementary Materials.

Human subjects

All experiments involving human subjects were carried out according to the institutional review board protocols approved by UCSD (project no. 071032) and NJH (HS-2581). Informed consent was obtained from all subjects.

Screening for antimicrobial activity

Up to 84 individual isolated colonies of CoNS from each skin site were randomly picked and cultured in TSB at 37°C overnight. Each plate also contained wells with a nonantimicrobial strain of *S. epidermidis* (ATCC1457) as negative control and a potent antimicrobial strain of *S. hominis* (A9 strain) as positive control. The antimicrobial activity of sterile conditioned medium prepared from each CoNS culture was evaluated by mixing with 1×10^4 CFU of *S. aureus* (ATCC35556). Antimicrobial strains were defined as those that suppressed *S. aureus* growth after 22 hours to less than 50% (I_{50}) of growth seen in negative controls.

Transplantation of antimicrobial CoNS on mice and ex situ pigskin

All experiments involving live animal work were in accordance with the approval of the Institutional Animal Care and Use Guidelines of the University of California, San Diego (protocol no. S09074). Fresh-frozen pigskin was purchased as sheets and was sanitized by

3% chloroxylenol and rinsed with sterile phosphate-buffered saline. For mouse experiments, the backs of female C57BL6 mice (6 weeks old) were shaved. *S. aureus* was then epicutaneously applied on the dorsal skin of mice or pigskin at the indicated CFU density. *S. hominis* A9 strain or inactive strains were subsequently applied on the skin for 20 hours. Live bacteria were harvested by swabbing to measure *S. aureus* survival.

Autologous microbiome transplant

The approach of AMT for subjects with AD has been officially approved by the U.S. Food and Drug Administration, and this protocol has been filed as an investigational new drug application (UCSD approval no. 15786). This trial has been registered at ClinicalTrials.gov (NCT01959113). AD subjects who are *S. aureus* carriers on the lesional sites of both antecubital fossa were screened. CoNS strains with anti–*S. aureus* activity were screened from the nonlesional site and identified by 16*S* rRNA sequencing. Each CoNS strain was expanded in TSB overnight and formulated at 1×10^7 CFU/g in Cetaphil lotion (Galderma). When multiple strains were included, an equal CFU of each CoNS was formulated at a total concentration of 1×10^7 CFU/g. One arm was treated with AMT formulation to get CoNS (1×10^5 CFU/cm^2) on the target skin. The other arm received an equal amount of moisturizer only. *S. aureus* CFU was measured before and 24 hours after transplant.

Statistical analysis

Paired *t* tests (two-tailed) were used to compare lesional to nonlesional samples within AD subjects, and independent *t* tests (two-tailed) were used to compare non-AD to AD samples. For nonnormally distributed variables such as CoNS with *Sh*-antibiotic-α frequency, nonparametric approaches such as Wilcoxon-Mann-Whitney tests for non-AD to AD samples and Wilcoxon signed-rank tests for lesional to nonlesional samples within AD subjects were used. Longitudinal mixed models of frequency of antimicrobial CoNS and the ratio of live *Staphylococcus* to *Staphylococcus* DNA over time were also fit. Each model included lesion type, visit, and their interaction term as fixed effects, whereas a compound symmetry structure was used to account for correlation between samples obtained from the same subject at multiple time points. Frequency of antimicrobial CoNS used a cumulative logit link and multinomial distribution of categorized percentages (≤20, 21–79, ≥80) to account for a bimodal distribution. Statistical analyses were performed using SAS software (version 9.3) and R software (version 3.1.1).

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/9/378/eaah4680/DC1

Methods

Fig. S1. Analysis of the skin bacterial community by 16*S* rRNA gene sequencing and its relationship to the capacity to culture *S. aureus* from the skin.

Fig. S2. Abundance of live *Staphylococcus* and *Staphylococcus*-specific DNA on the skin of non-AD and AD subjects.

Fig. S3. Specificity of *Staphylococcus* genus–specific primers for real-time qPCR.

Fig. S4. Anti–*S. aureus* activity in CoNS is stable over time.

Fig. S5. Effect of vehicle treatment on the survival of *S. aureus* on ex situ pigskin or live mouse skin.

Fig. S6. MALDI-TOF MS analysis for two AMPs purified from *S. hominis*.

Fig. S7. Representation of amino acid losses in genome-guided MALDI-TOF/TOF analysis for *Sh*-lantibiotic-β.

Fig. S8. Skin isolate strains of CoNS exert selective antimicrobial activity against *S. aureus*.

Fig. S9. Detection of *Sh*-lantibiotic-α peptide by Western blotting from extracts of skin swabs taken from two non-AD subjects and two AD subjects.

Fig. S10. List of hypothetical antimicrobial genes identified in anti–*S. aureus* CoNS clones used for AMT.

Fig. S11. List of previously known lantibiotic genes identified in antimicrobial *S. epidermidis* clones isolated from non-AD skin.

Fig. S12. Study design of AMT therapy for patients with AD.

Fig. S13. Effect of transplantation of antimicrobial CoNS or vehicle on the survival of *S. aureus* on the skin of five subjects with AD.

Table S1. Clinical characteristics of AD and non-AD subjects.

Table S2. Proportion of CoNS species identified with antimicrobial and nonantimicrobial activity.

Table S3. Clinical characteristics of AD subjects receiving AMT.

Table S4. Sequences of PCR primers.

Table S5. Primary data (provided as an Excel file).

References (*45*–*63*)

REFERENCES AND NOTES

1. T. Nakatsuji, R. L. Gallo, Antimicrobial peptides: Old molecules with new ideas. *J. Invest. Dermatol.* **132**, 887–895 (2012).
2. R. L. Gallo, L. V. Hooper, Epithelial antimicrobial defence of the skin and intestine. *Nat. Rev. Immunol.* **12**, 503–516 (2012).
3. L.-j. Zhang, C. F. Guerrero-Juarez, T. Hata, S. P. Bapat, R. Ramos, M. V. Plikus, R. L. Gallo, Innate immunity. Dermal adipocytes protect against invasive *Staphylococcus aureus* skin infection. *Science* **347**, 67–71 (2015).
4. H. C. Clevers, C. L. Bevins, Paneth cells: Maestros of the small intestinal crypts. *Annu. Rev. Physiol.* **75**, 289–311 (2013).
5. N. N. Schommer, R. L. Gallo, Structure and function of the human skin microbiome. *Trends Microbiol.* **21**, 660–668 (2013).
6. E. A. Grice, H. H. Kong, S. Conlan, C. B. Deming, J. Davis, A. C. Young; NISC Comparative Sequencing Program, G. G. Bouffard, R. W. Blakesley, P. R. Murray, E. D. Green, M. L. Turner, J. A. Segre, Topographical and temporal diversity of the human skin microbiome. *Science* **324**, 1190–1192 (2009).
7. H. H. Kong, J. Oh, C. Deming, S. Conlan, E. A. Grice, M. A. Beatson, E. Nomicos, E. C. Polley, H. D. Komarow; NISC Comparative Sequence Program, P. R. Murray, M. L. Turner, J. A. Segre, Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* **22**, 850–859 (2012).
8. J. J. Leyden, R. R. Marples, A. M. Kligman, *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br. J. Dermatol.* **90**, 525–530 (1974).
9. L. Mallbris, L. Carlén, T. Wei, J. Heilborn, M. F. Nilsson, F. Granath, M. Ståhle, Injury downregulates the expression of the human cathelicidin protein hCAP18/LL-37 in atopic dermatitis. *Exp. Dermatol.* **19**, 442–449 (2010).
10. P. Y. Ong, T. Ohtake, C. Brandt, I. Strickland, M. Boguniewicz, T. Ganz, R. L. Gallo, D. Y. M. Leung, Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N. Engl. J. Med.* **347**, 1151–1160 (2002).
11. T. R. Hata, P. Kotol, M. Boguniewicz, P. Taylor, A. Paik, M. Jackson, M. Nguyen, F. Kabigting, J. Miller, M. Gerber, D. Zaccaro, B. Armstrong, R. Dorschner, D. Y. M. Leung, R. L. Gallo, History of eczema herpeticum is associated with the inability to induce human β-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. *Br. J. Dermatol.* **163**, 659–661 (2010).
12. M. D. Howell, M. Boguniewicz, S. Pastore, N. Novak, T. Bieber, G. Girolomoni, D. Y. M. Leung, Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin. Immunol.* **121**, 332–338 (2006).
13. M. D. Howell, R. L. Gallo, M. Boguniewicz, J. F. Jones, C. Wong, J. E. Streib, D. Y. M. Leung, Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. *Immunity* **24**, 341–348 (2006).
14. Z. Allakhverdi, M. R. Comeau, H. K. Jessup, B.-R. P. Yoon, A. Brewer, S. Chartier, N. Paquette, S. F. Ziegler, M. Sarfati, G. Delespesse, Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J. Exp. Med.* **204**, 253–258 (2007).
15. M. Sieprawska-Lupa, P. Mydel, K. Krawczyk, K. Wojcik, M. Puklo, B. Lupa, P. Suder, J. Silberring, M. Reed, J. Pohl, W. Shafer, F. McAleese, T. Foster, J. Travis, J. Potempa, Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. *Antimicrob. Agents Chemother.* **48**, 4673–4679 (2004).
16. A. T. Vu, T. Baba, X. Chen, T. A. Le, H. Kinoshita, Y. Xie, S. Kamijo, K. Hiramatsu, S. Ikeda, H. Ogawa, K. Okumura, T. Takai, *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2–Toll-like receptor 6 pathway. *J. Allergy Clin. Immunol.* **126**, 985–993.e3 (2010).
17. Y. Nakamura, J. Oscherwitz, K. B. Cease, S. M. Chan, R. Muñoz-Planillo, M. Hasegawa, A. E. Villaruz, G. Y. C. Cheung, M. J. McGavin, J. B. Travers, M. Otto, N. Inohara, G. Núñez, *Staphylococcus* δ-toxin induces allergic skin disease by activating mast cells. *Nature* **503**, 397–401 (2013).

18. L. S. Miller, J. S. Cho, Immunity against *Staphylococcus aureus* cutaneous infections. *Nat. Rev. Immunol.* **11**, 505–518 (2011).
19. T. Kobayashi, M. Glatz, K. Horiuchi, H. Kawasaki, H. Akiyama, D. H. Kaplan, H. H. Kong, M. Amagai, K. Nagao, Dysbiosis and *Staphylococcus aureus* colonization drives inflammation in atopic dermatitis. *Immunity* **42**, 756–766 (2015).
20. T. Nakatsuji, T. H. Chen, A. M. Two, K. A. Chun, S. Narala, R. S. Geha, T. R. Hata, R. L. Gallo, *Staphylococcus aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression. *J. Invest. Dermatol.* **136**, 2192–2200 (2016).
21. R. L. Gallo, T. Nakatsuji, Microbial symbiosis with the innate immune defense system of the skin. *J. Invest. Dermatol.* **131**, 1974–1980 (2011).
22. Y. Lai, A. Di Nardo, T. Nakatsuji, A. Leichtle, Y. Yang, A. L. Cogen, Z.-R. Wu, L. V. Hooper, R. R. Schmidt, S. von Aulock, K. A. Radek, C.-M. Huang, A. F. Ryan, R. L. Gallo, Commensal bacteria regulate Toll-like receptor 3–dependent inflammation after skin injury. *Nat. Med.* **15**, 1377–1382 (2009).
23. S. Naik, N. Bouladoux, J. L. Linehan, S.-J. Han, O. J. Harrison, C. Wilhelm, S. Conlan, S. Himmelfarb, A. L. Byrd, C. Deming, M. Quinones, J. M. Brenchley, H. H. Kong, R. Tussiwand, K. M. Murphy, M. Merad, J. A. Segre, Y. Belkaid, Commensal–dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* **250**, 104–108 (2015).
24. S. Naik, N. Bouladoux, C. Wilhelm, M. J. Molloy, R. Salcedo, W. Kastenmuller, C. Deming, M. Quinones, L. Koo, S. Conlan, S. Spencer, J. A. Hall, A. Dzutsev, H. Kong, D. J. Campbell, G. Trinchieri, J. A. Segre, Y. Belkaid, Compartmentalized control of skin immunity by resident commensals. *Science* **337**, 1115–1119 (2012).
25. E. Laborel-Preneron, P. Bianchi, F. Boralevi, P. Lehours, F. Fraysse, F. Morice-Picard, M. Sugai, Y. Sato'o, C. Badiou, G. Lina, A. M. Schmitt, D. Redoules, C. Casas, C. Davrinche, Effects of the *Staphylococcus aureus* and *Staphylococcus epidermidis* secretomes isolated from the skin microbiota of atopic children on $CD4^+$ T cell activation. *PLOS ONE* **10**, e0144323 (2015).
26. Y. Lai, A. L. Cogen, K. A. Radek, H. J. Park, D. T. Macleod, A. Leichtle, A. F. Ryan, A. Di Nardo, R. L. Gallo, Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. *J. Invest. Dermatol.* **130**, 2211–2221 (2010).
27. I. Wanke, H. Steffen, C. Christ, B. Krismer, F. Götz, A. Peschel, M. Schaller, B. Schittek, Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *J. Invest. Dermatol.* **131**, 382–390 (2011).
28. D. Li, H. Lei, Z. Li, H. Li, Y. Wang, Y. Lai, A novel lipopeptide from skin commensal activates TLR2/CD36-p38 MAPK signaling to increase antibacterial defense against bacterial infection. *PLOS ONE* **8**, e58288 (2013).
29. A. L. Cogen, K. Yamasaki, J. Muto, K. M. Sanchez, L. Crotty Alexander, J. Tanios, Y. Lai, J. E. Kim, V. Nizet, R. L. Gallo, *Staphylococcus epidermidis* antimicrobial δ-toxin (phenol-soluble modulin-γ) cooperates with host antimicrobial peptides to kill group A *Streptococcus*. *PLOS ONE* **5**, e8557 (2010).
30. A. L. Cogen, K. Yamasaki, K. M. Sanchez, R. A. Dorschner, Y. Lai, D. T. MacLeod, J. W. Torpey, M. Otto, V. Nizet, J. E. Kim, R. L. Gallo, Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. *J. Invest. Dermatol.* **130**, 192–200 (2010).
31. E. Rubinchik, D. Dugourd, T. Algara, C. Pasetka, H. D. Friedland, Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models. *Int. J. Antimicrob. Agents* **34**, 457–461 (2009).
32. S. Jiang, B. Zheng, W. Ding, L. Lv, J. Ji, H. Zhang, Y. Xiao, L. Li, Whole-genome sequence of *Staphylococcus hominis*, an opportunistic pathogen. *J. Bacteriol.* **194**, 4761–4762 (2012).
33. O. McAuliffe, R. P. Ross, C. Hill, Lantibiotics: Structure, biosynthesis and mode of action. *FEMS Microbiol. Rev.* **25**, 285–308 (2001).
34. M. B. C. Fontana, M. d. C. de Bastos, A. Brandelli, Bacteriocins Pep5 and epidermin inhibit *Staphylococcus epidermidis* adhesion to catheters. *Curr. Microbiol.* **52**, 350–353 (2006).
35. R. A. Dorschner, K. H. Lin, M. Murakami, R. L. Gallo, Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: Innate immunity during development of the adaptive response. *Pediatr. Res.* **53**, 566–572 (2003).
36. R. Lande, J. Gregorio, V. Facchinetti, B. Chatterjee, Y.-H. Wang, B. Homey, W. Cao, Y.-H. Wang, B. Su, F. O. Nestle, T. Zal, I. Mellman, J.-M. Schröder, Y.-J. Liu, M. Gilliet, Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* **449**, 564–569 (2007).
37. V. Nizet, T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, R. L. Gallo, Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* **414**, 454–457 (2001).
38. I. A. Myles, K. W. Williams, J. D. Reckhow, M. L. Jammeh, N. B. Pincus, I. Sastalla, D. Saleem, K. D. Stone, S. K. Datta, Transplantation of human skin microbiota in models of atopic dermatitis. *JCI Insight* **1**, e86955 (2016).
39. T. Iwase, Y. Uehara, H. Shinji, A. Tajima, H. Seo, K. Takada, T. Agata, Y. Mizunoe, *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* **465**, 346–349 (2010).
40. S. Kommineni, D. J. Bretl, V. Lam, R. Chakraborty, M. Hayward, P. Simpson, Y. Cao, P. Bousounis, C. J. Kristich, N. H. Salzman, Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* **526**, 719–722 (2015).
41. J. Oh, A. L. Byrd, C. Deming, S. Conlan; NISC Comparative Sequencing Program, H. H. Kong, J. A. Segre, Biogeography and individuality shape function in the human skin metagenome. *Nature* **514**, 59–64 (2014).
42. D. Kang, B. Shi, M. C. Erfe, N. Craft, H. Li, Vitamin B_{12} modulates the transcriptome of the skin microbiota in acne pathogenesis. *Sci. Transl. Med.* **7**, 293ra103 (2015).
43. A. Bouslimani, C. Porto, C. M. Rath, M. Wang, Y. Guo, A. Gonzalez, D. Berg-Lyon, G. Ackermann, G. J. Moeller Christensen, T. Nakatsuji, L. Zhang, A. W. Borkowski, M. J. Meehan, K. Dorrestein, R. L. Gallo, N. Bandeira, R. Knight, T. Alexandrov, P. C. Dorrestein, Molecular cartography of the human skin surface in 3D. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E2120–E2129 (2015).
44. R. Kaddurah-Daouk, R. A. Baillie, H. Zhu, Z.-B. Zeng, M. M. Wiest, U. T. Nguyen, K. Wojnoonski, S. M. Watkins, M. Trupp, R. M. Krauss, Enteric microbiome metabolites correlate with response to simvastatin treatment. *PLOS ONE* **6**, e25482 (2011).
45. D. W. Fadrosh, B. Ma, P. Gajer, N. Sengamalay, S. Ott, R. M. Brotman, J. Ravel, An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome* **2**, 6 (2014).
46. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010).
47. R. C. Edgar, Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010).
48. T. Z. DeSantis, P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, G. L. Andersen, Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72**, 5069–5072 (2006).
49. R. C. Edgar, B. J. Haas, J. C. Clemente, C. Quince, R. Knight, UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194–2200 (2011).
50. Q. Wang, G. M. Garrity, J. M. Tiedje, J. R. Cole, Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267 (2007).
51. J. N. Paulson, O. C. Stine, H. C. Bravo, M. Pop, Differential abundance analysis for microbial marker-gene surveys. *Nat. Methods* **10**, 1200–1202 (2013).
52. C. H. Carter, Egg yolk agar for isolation of coagulase-positive staphylococci. *J. Bacteriol.* **79**, 753–754 (1960).
53. J. T. Parisi, B. H. Hamory, Simplified method for the isolation, identification, and characterization of *Staphylococcus epidermidis* in epidemiologic studies. *Diagn. Microbiol. Infect. Dis.* **4**, 29–35 (1986).
54. J. R. Cole, Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, J. M. Tiedje, Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **42**, D633–D642 (2014).
55. R. D. Kersten, Y.-L. Yang, Y. Xu, P. Cimermancic, S.-J. Nam, W. Fenical, M. A. Fischbach, B. S. Moore, P. C. Dorrestein, A mass spectrometry–guided genome mining approach for natural product peptidogenomics. *Nat. Chem. Biol.* **7**, 794–802 (2011).
56. M. H. Medema, K. Blin, P. Cimermancic, V. de Jager, P. Zakrzewski, M. A. Fischbach, T. Weber, E. Takano, R. Breitling, antiSMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res.* **39**, W339–W346 (2011).
57. B. Li, D. Sher, L. Kelly, Y. Shi, K. Huang, P. J. Knerr, I. Joewono, D. Rusch, S. W. Chisholm, W. A. van der Donk, Catalytic promiscuity in the biosynthesis of cyclic peptide secondary metabolites in planktonic marine cyanobacteria. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 10430–10435 (2010).
58. A. Tanca, A. Palomba, M. Deligios, T. Cubeddu, C. Fraumene, G. Biosa, D. Pagnozzi, M. F. Addis, S. Uzzau, Evaluating the impact of different sequence databases on metaproteome analysis: Insights from a lab-assembled microbial mixture. *PLOS ONE* **8**, e82981 (2013).
59. M. Zaiou, V. Nizet, R. L. Gallo, Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J. Invest. Dermatol.* **120**, 810–816 (2003).
60. S. M. Paule, A. C. Pasquariello, D. M. Hacek, A. G. Fisher, R. B. Thomson Jr., K. L. Kaul, L. R. Peterson, Direct detection of *Staphylococcus aureus* from adult and neonate nasal swab specimens using real-time polymerase chain reaction. *J. Mol. Diagn.* **6**, 191–196 (2004).
61. T. Iwase, S. Hoshina, K. Seki, H. Shinji, S. Masuda, Y. Mizunoe, Rapid identification and specific quantification of *Staphylococcus epidermidis* by 5′ nuclease real-time polymerase chain reaction with a minor groove binder probe. *Diagn. Microbiol. Infect. Dis.* **60**, 217–219 (2008).

62. K. Matsuda, H. Tsuji, T. Asahara, K. Matsumoto, T. Takada, K. Nomoto, Establishment of an analytical system for the human fecal microbiota, based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules. *Appl. Environ. Microbiol.* **75**, 1961–1969 (2009).
63. H. Jiang, H. Dong, G. Zhang, B. Yu, L. R. Chapman, M. W. Fields, Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. *Appl. Environ. Microbiol.* **72**, 3832–3845 (2006).

Funding: This work was funded by the Atopic Dermatitis Research Network (ADRN) (NIH/NIAID 1 U19 AI117673-02 to R.L.G. and D.Y.M.L.), the Rho Federal Systems Division, and NIH grants (R21AR067547, R01AR06781, R01AI052453, and R01AI118816 to R.L.G.). S.N. is supported by an NIH grant (1TL1TR001443). **Author contributions:** T.N. and R.L.G. conceived and designed this study and wrote the manuscript. T.R.H., A.M.T., K.A.C., T.Y., F.S., P.T., J.S., D.Y.M.L., and R.L.G. procured clinical samples. K.A. and G.D. coordinated the clinical study. T.N., T.H.C., S.N., and P.F.K. analyzed data from clinical samples. T.N., A.B., A.V.M., and P.C.D. assisted in the identification of AMPs. H.L., J.-N.K., and K.Z. performed genome sequencing and analysis of CoNS strains. A.G. and S.R.G. conducted microbiome 16*S* rRNA sequencing. T.N., R.L.G., A.L., and G.D. evaluated and analyzed the data and conducted statistical analysis. D.Y.M.L. and R.L.G. directed this project as the principal investigators of ADRN. **Competing interests:** T.N. and R.L.G. are co-inventors of UCSD technology related to the bacterial AMPs discussed herein, and R.L.G. is a cofounder and consultant of MatriSys Bioscience (La Jolla, CA), a company that is developing skin bacteriotherapy. **Data and materials availability:** The 16*S* rRNA sequence data for this study have been published in the DDBJ Sequence Read Archive (http://trace.ddbj.nig.ac.jp/dra/index_e.html) under the accession code DRA005329.

Submitted 30 June 2016
Resubmitted 8 October 2016
Accepted 17 January 2017
Published 22 February 2017
10.1126/scitranslmed.aah4680

Citation: T. Nakatsuji, T. H. Chen, S. Narala, K. A. Chun, A. M. Two, T. Yun, F. Shafiq, P. F. Kotol, A. Bouslimani, A. V. Melnik, H. Latif, J.-N. Kim, A. Lockhart, K. Artis, G. David, P. Taylor, J. Streib, P. C. Dorrestein, A. Grier, S. R. Gill, K. Zengler, T. R. Hata, D. Y. M. Leung, R. L. Gallo, Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci. Transl. Med.* **9**, eaah4680 (2017).

Science Translational Medicine

Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis

Teruaki Nakatsuji, Tiffany H. Chen, Saisindhu Narala, Kimberly A. Chun, Aimee M. Two, Tong Yun, Faiza Shafiq, Paul F. Kotol, Amina Bouslimani, Alexey V. Melnik, Haythem Latif, Ji-Nu Kim, Alexandre Lockhart, Keli Artis, Gloria David, Patricia Taylor, Joanne Streib, Pieter C. Dorrestein, Alex Grier, Steven R. Gill, Karsten Zengler, Tissa R. Hata, Donald Y. M. Leung and Richard L. Gallo

Sci Transl Med **9**, eaah4680.
DOI: 10.1126/scitranslmed.aah4680

Bacterial biological warfare in atopic dermatitis

Normal human skin is colonized by a variety of bacteria, which typically do not perturb the host. However, *Staphylococcus aureus* is known to aggravate symptoms of atopic dermatitis. Nakatsuji *et al*. report that other strains of *Staphylococcus* residing on the skin of healthy individuals produce a novel antimicrobial peptide that can inhibit *S. aureus* growth. Colonization of pigskin or mice with these protective commensals reduced *S. aureus* replication. Autologous bacterial transplant in a small number of atopic dermatitis patients drastically reduced *S. aureus* skin burden. This commensal skin transplant is already approved by the U.S. Food and Drug Administration, with a clinical trial underway.