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CONCISE COMMUNICATION

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The influence of the commensal skin bacterium *Staphylococcus epidermidis* on the epidermal barrier and inflammation: Implications for atopic dermatitis

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Abstract

The skin microbiota is a crucial component in maintaining cutaneous barrier function. *Staphylococcus epidermidis* is considered as a beneficial commensal member of the cutaneous microbiota promoting skin health. However, *S. epidermidis* is also frequently detectable in the skin of patients with the inflammatory skin disease atopic dermatitis (AD) and some studies reported a significantly higher presence of *S. epidermidis* in severe AD as compared to mild AD. Therefore, this study aimed to analyse the impact of *S. epidermidis* on the expression of cutaneous inflammatory mediators and skin barrier molecules. Various *S. epidermidis* skin-derived isolates activated the proinflammatory transcription factor NF-kappaB and induced expression of AD-associated proinflammatory cytokines in human primary keratinocytes and 3D skin equivalents. Skin barrier molecules such as filaggrin were downregulated by *S. epidermidis*. In general, AD-derived *S. epidermidis* strains elicited a higher response than strains derived from the skin of healthy individuals. Taken together, our results provide further evidence that the abundance of *S. epidermidis* in AD may trigger the inflammatory scenario associated with this disease.

KEYWORDS
atopic dermtitis, keratinocytes, skin inflammation, *Staphylococcus epidermidis*

1 | BACKGROUND

Coagulase-negative *Staphylococci* (CoNS) like *Staphylococcus* (*S.*) *epidermidis* are abundant commensal members of the skin microbiota. Although CoNS can cause skin and soft tissue infections[1] they are generally believed to be beneficial for the cutaneous barrier. For example, distinct skin-derived CoNS such as *S. epidermidis* and *Staphylococcus hominis* release specific antimicrobial peptides (bacteriocins) that selectively kill *Staphylococcus aureus*, a major skin-related pathogen commonly isolated from the skin of patients with inflammatory skin disease atopic dermatitis (AD). These *S. aureus*-killing CoNS strains are rare in AD and application of such antimicrobial CoNS onto the AD skin reduced *S. aureus* load.[2] In addition, specific CoNS communicate with each other by the release of autoinducing peptides that inhibit the virulence and skin-damaging properties of *S. aureus*.[3,4]

As an abundant commensal member of human skin, one would speculate that *S. epidermidis* in contrast to the skin pathogen *S. aureus*

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does not exhibit distinct proinflammatory activities by induction of proinflammatory mediators. Concurrent with that speculation it has been reported that in contrast to the *S. aureus*, the *S. epidermidis* strain did not elicit a significant induction of proinflammatory cytokines in a 3D skin equivalent.[5] However, other studies have shown that *S. epidermidis* induced the expression of proinflammatory cytokines such as IL-1 in keratinocytes.[6–8] This strengthens the hypothesis that strains of *S. epidermidis* may also have proinflammatory properties by induction of proinflammatory mediators in keratinocytes. Such proinflammatory activities may help to explain an observed correlation between *S. epidermidis* abundance and severity of AD.[9,10]

2 | QUESTION ADDRESSED

This study was initiated to investigate the ability of *S. epidermidis* to induce AD-relevant proinflammatory factors in keratinocytes and 3D skin equivalents to get more insight into the pathophysiological aspects of microbiota in AD.

3 | EXPERIMENTAL DESIGN

The study was performed with monolayers and 3D skin equivalents of primary normal human epidermal keratinocytes (NHEKs). Monolayer cells of NHEKs were transfected with an NF-kappaB *firefly* luciferase gene reporter and a *renilla* luciferase control plasmid and treated with different culture supernatants of AD-patient-isolated *S. epidermidis* for 6 h. After cell lysis luciferase activity of *firefly* and *renilla* was measured.

Other monolayer cells of NHEKs were exposed to culture supernatants of *S. epidermidis* from healthy or AD donors for 6 h. Gene expression of proinflammatory markers was measured by quantitative real-time polymerase chain reaction (PCR).

3D skin equivalents were treated with different living *S. epidermidis* strains isolated from healthy or AD donors, respectively. After 22–24 h incubation either immunostaining, ELISA or quantitative real-time PCR was performed.

The isolation of *S. epidermidis* strains by rinsing lesions of AD patients or the skin of healthy volunteers was approved by the Ethical Committee of the Medical Faculty at Kiel University (A 104/06). All study participants gave their written consent.

Detailed information on methods and material are embedded in Appendix S1.

4 | RESULTS

4.1 | *S. epidermidis* activates NF-kappaB in NHEKs

To analyse whether *S. epidermidis* has the capacity to activate the inflammation-associated transcription factor NF-kappaB, NHEKs were transfected with an NF-kappaB luciferase gene reporter plasmid and subsequently stimulated with culture supernatants of seven different *S. epidermidis* strains isolated from the lesions of atopic dermatitis patients' skin. This revealed a significant induction of luciferase activity by four strains indicating activation of NF-kappaB (Figure 1). A non-significant increase by the other three strains was also observed. These data show that various *S. epidermidis* strains have the capacity to activate NF-kappaB.

4.2 | *S. epidermidis* induces proinflammatory cytokines in NHEKs

Based on the observation that *S. epidermidis* activates NF-kappaB we hypothesized that *S. epidermidis* may induce proinflammatory cytokines in keratinocytes. To address this hypothesis we performed a 2D monolayer NHEK stimulation assay with culture supernatants of 11 different *S. epidermidis* strains derived from lesions of atopic dermatitis patients' skin. This revealed a significant upregulation of gene expression of TSLP, IL-17C and TNF-alpha. *S. epidermidis* strains from healthy individuals' skin revealed also a significant induction of these inflammatory mediators with no significant difference from atopic dermatitis-derived strains (Figure 2). Gene expression of the NF-kappaB inhibitor IkappaBalpha (NFKBIA) was also induced by *S. epidermidis* (Figure 2) confirming NF-kappaB activation because activation of NF-kappaB correlates with gene induction of NFKBIA.[11] A Spearman's rank correlation analysis revealed a significant

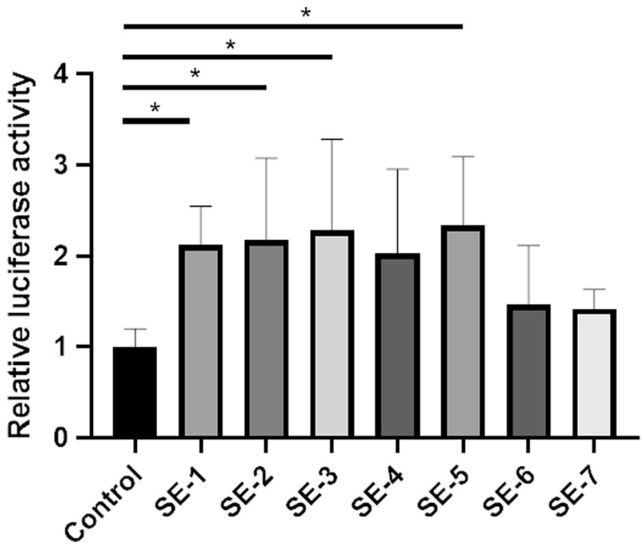

FIGURE 1 *S. epidermidis* activates NF-kappaB in NHEKs. NHEKs transfected with an NF-kappaB *firefly* luciferase gene reporter plasmid and a *renilla* luciferase control plasmid were treated with culture supernatants of seven atopic dermatitis-derived *S. epidermidis* isolates (SE-1-7) for 6 h. After stimulation cells were lysed and NF-kappaB activity was determined as a quotient of *firefly* and *renilla* luciferase activity. Bars are means + SEM ($n = 6$, $^*p < 0.05$; Kruskal–Wallis test with Dunn's multiple comparisons test).

expression correlation of NFKBIA with TSLP (correlation coefficient 0.818, $p = 0.003$) and TNF-alpha (correlation coefficient 0.727, $p = 0.014$). This suggests that NF-kappaB activation by distinct *S. epidermidis* strains is associated with an increased capacity to induce proinflammatory mediators such as TSLP and TNF-alpha.

4.3 | *S. epidermidis* induces proinflammatory cytokines and inhibits the expression of barrier molecules in human 3D skin equivalents

To evaluate the capability of *S. epidermidis* to induce proinflammatory cytokines in a differentiated epidermis, 3D skin equivalents were generated and treated with living *S. epidermidis*. Stimulation of the skin equivalents with *S. epidermidis* derived from atopic dermatitis skin significantly induced gene expression of TSLP, TNF-alpha, IL-17C, IL-1alpha, IL-1beta and IL-36gamma (Figure 3A). In concordance with the observed NF-kappaB activation in cultured primary keratinocytes, NFKBIA was also induced by *S. epidermidis* derived from atopic dermatitis. Of note, except for IL-36gamma *S. epidermidis* strains derived from healthy skin revealed less induction and TSLP, IL-1beta and NFKBIA gene expression were not significantly induced by healthy skin-derived *S. epidermidis* (Figure 3A). In line with the gene expression data, protein release of TSLP and TNF-alpha was significantly induced by *S. epidermidis* from atopic dermatitis skin but not by healthy skin-derived *S. epidermidis* as measured by ELISA (Figure 3B). Moreover, TSLP immunohistochemistry of a 3D skin equivalent stimulated with *S. epidermidis* showed an increased TSLP immune reactivity with higher intensity elicited by an atopic dermatitis-derived *S. epidermidis* strain as compared to a strain from healthy skin (Figure 3C). Gene expression of the Th2-related factors IL-4, IL-5, IL-10, IL-13 and CCL17 was not detectable. Treatment of 3D skin with alive *S. epidermidis* revealed a significant downregulation of filaggrin gene expression by atopic dermatitis-derived strains and strains derived from healthy skin (Figure 3D). *S. epidermidis* led also to a decreased expression of keratin 1 and keratin 10 but only atopic dermatitis-derived *S. epidermidis* revealed significant downregulation of both keratin molecules (Figure 3D). In concordance with the gene expression data, filaggrin immunostaining of 3D skin models treated with *S. epidermidis* revealed a reduced filaggrin immunoreactivity (Figure 3E).

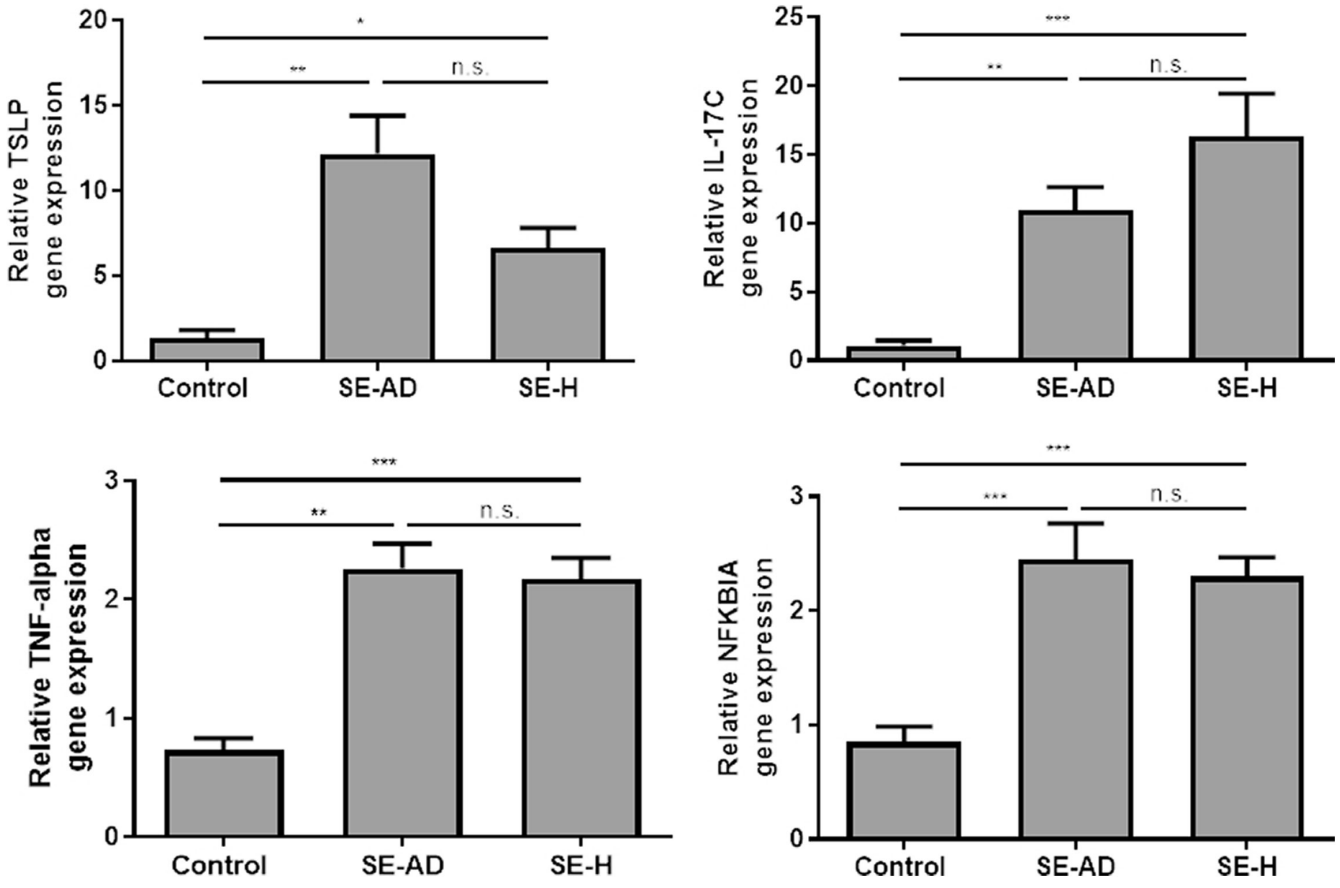

FIGURE 2 *S. epidermidis* activates expression of proinflammatory mediators in NHEKs. NHEKs were exposed for 6 h to culture supernatants of *S. epidermidis* derived from either lesional atopic dermatitis skin (SE-AD, $n = 11$) or skin of healthy individuals (SE-H, $n = 7$). Relative expression of different proinflammatory genes was determined by quantitative real-time PCR. Bars are means + SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s., not significant; Kruskal–Wallis test with Dunn's multiple comparisons test).

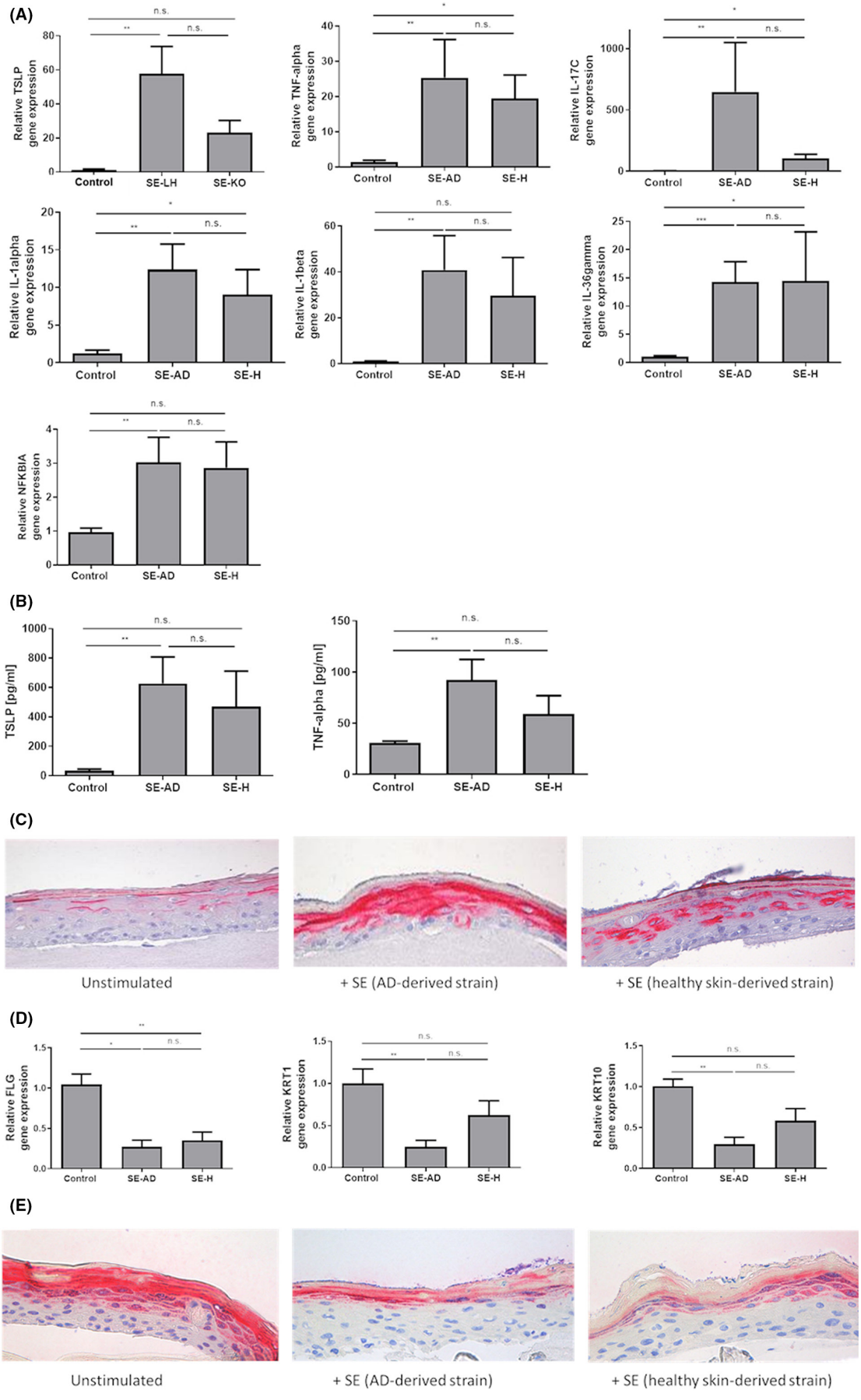

FIGURE 3 *S. epidermidis* induces expression of proinflammatory mediators and down-regulates expression of differentiation marker in 3D skin equivalents. (A, B, D) 3D skin equivalents were treated for 22–24 h with alive *S. epidermidis* isolated from either lesional atopic dermatitis skin (SE-AD, $n = 9$) or skin of healthy individuals (SE-H, $n = 9$). (A, D) Gene expression of various proinflammatory and differentiation markers was measured by quantitative real-time PCR. (B) TSLP and TNF-alpha protein secretion was measured by ELISA. Bars are means + SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, n.s., not significant; Friedman test with Dunn's multiple comparisons test). (C, E) Immunostaining of TSLP (C) and filaggrin (E) in 3D skin equivalents stimulated with *S. epidermidis* derived from lesional atopic dermatitis skin or healthy skin.

5 | CONCLUSIONS AND PERSPECTIVES

Staphylococcus epidermidis is an important member of the skin microbiota and in general believed to be beneficial for the host. It produces antimicrobial factors such as bacteriocins that exhibit selective antimicrobial action against skin pathogens like *S. aureus*.[2,12,13] *S. epidermidis* induces also the expression of antimicrobial peptides in keratinocytes.[7,14–16] As a member of the commensal cutaneous microbiota one may expect no proinflammatory traits of *S. epidermidis*. In line with that and in contrast to our data a recent study reported no induction of proinflammatory cytokines in a skin equivalent treated with *S. epidermidis*.[5] However, that study used only one *S. epidermidis* strain derived from healthy forearm skin. Our data reveal that various isolates of *S. epidermidis* have the capacity to induce proinflammatory cytokines in a 3D skin equivalent. This induction was shown to be associated with an activation of the inflammation-related transcription factor NF-kappaB. One may hypothesize that the activation of proinflammatory mediators by *S. epidermidis* is a prerequisite for activating cutaneous innate immunity on a subclinical level. In line with that, it has been shown that skin colonization of germ-free mice with *S. epidermidis* increased cutaneous protection against infection and stimulation of keratinocytes and dendritic cells by *S. epidermidis*-activated IL-1-mediated immune reactions.[6,17]

Given that *S. epidermidis* is abundant on healthy skin it is likely that the induction of pro-inflammatory mediators in keratinocytes by *S. epidermidis* should be tightly regulated to prevent the induction of excessive inflammatory events. In line with that hypothesis, it has been reported that *S. epidermidis* exerts anti-inflammatory activity in the skin through lipoteichoic acids and lipopeptides.[18,19] Moreover, *S. epidermidis* induced the expression of the host protein A20 in keratinocytes. A20 functions as an inhibitor of the NF-kappaB signalling pathway thereby controlling the expression of proinflammatory cytokines.[8] Thus, it is likely that the parallel induction of A20 by *S. epidermidis* controls *S. epidermidis*-mediated induction of proinflammatory cytokines.

S. aureus as a skin pathogen is often associated with inflammation as seen in atopic dermatitis where *S. aureus* is believed to be a driver of inflammation.[20] In contrast to *S. aureus*, *S. epidermidis* as an abundant skin commensal is not typically considered an atopic dermatitis-associated bacterium. However, skin microbiome studies using sequencing and PCR techniques revealed an elevated abundance of *S. epidermidis* in the skin of atopic dermatitis patients.[6,10,21–23] Moreover, a correlation between a load of *S. epidermidis* and severity of atopic dermatitis has been reported.[9,10] This may suggest that specific *S. epidermidis* strains may trigger atopic dermatitis-associated barrier disruption and inflammation under certain conditions. In support of this hypothesis, it has been recently shown that the presence of *S. epidermidis* and the production of its protease EcpA, which caused skin barrier disruption, correlated with the disease severity of atopic dermatitis.[24] Our study revealed that protein expression of the atopic dermatitis-associated cytokines TSLP and TNF-alpha were only significantly induced in a 3D skin equivalent by *S. epidermidis* strains derived from lesional atopic dermatitis skin whereas the induction by *S. epidermidis* derived from healthy skin reached no statistical significance. This leads to the hypothesis that *S. epidermidis* may trigger inflammation in lesional atopic skin through two mechanisms: on the one hand due to the high abundance of *S. epidermidis* in atopic dermatitis as mentioned above and on the other hand due to the atopic dermatitis-related presence of strains with a higher capacity to induce proinflammatory mediators, a hypothesis which remains to be verified in future studies. In addition, it remains to be shown how the atopic dermatitis-related cytokine milieu may influence the *S. epidermidis*–keratinocytes interaction. Besides atopic dermatitis, *S. epidermidis* is frequently isolated from sites of various lesional infectious and inflammatory skin diseases suggesting that *S. epidermidis* may contribute to the inflammatory process in different skin diseases.[25]

It remains to be determined which factor(s) of *S. epidermidis* elicit the observed induction of proinflammatory cytokines in keratinocytes. In addition to classical microbial-associated molecular patterns such as lipoproteins, lipoteichoic acid and peptidoglycan fragments that serve as ligands for pattern recognition receptors, an additional candidate may be phenol-soluble modulins (PSMs). PSMs are *S. epidermidis*-derived toxins that are associated with more aggressive and virulent behaviour of *S. epidermidis*.[26] PSMs activated and functioned as a chemoattractant factor for monocytes and neutrophils[27] and expression and proinflammatory traits of PSMs are controlled by bacterial quorum-sensing.[28] Thus, quorum-sensing may be associated with an increased capability of *S. epidermidis* to induce proinflammatory mediators in keratinocytes, a hypothesis that remains to be investigated.

In contrast to the induction of proinflammatory mediators, our data revealed also an *S. epidermidis*-mediated downregulation of the differentiation marker filaggrin, keratin 1 and keratin 10 in 3D skin equivalents. In contrast to the atopic dermatitis-derived *S. epidermidis* strains, the downregulation of keratin 1 and keratin 10 was not significantly downregulated by *S. epidermidis* strains derived from healthy skin. Thus, an increased occurrence of *S. epidermidis* may contribute to the known decreased expression of barrier molecules in atopic dermatitis[29,30] and therefore may trigger barrier disruption in this disease.

Taken together, we provide further evidence that *S. epidermidis* isolated from atopic dermatitis skin, but also many *S. epidermidis* strains in general, are able to induce proinflammatory mediators in skin. Thus, the proinflammatory properties of *S. epidermidis* together with its overabundance in many atopic dermatitis patients as well as the associated disease severity supports the hypothesis that *S. epidermidis* may trigger inflammation in atopic dermatitis and other inflammatory skin diseases.

AUTHOR CONTRIBUTIONS

DO, FR, RG and JH conceived and designed the experiments. DO, FR and KAD performed the experiments. DO, FR, RG and JH analysed the data. JH wrote the paper. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Natsis NE, Cohen PR. Coagulase-negative Staphylococcus skin and soft tissue infections. *Am J Clin Dermatol*. 2018;19(5):671-677.
2. Nakatsuji T, Chen TH, Narala S, et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med*. 2017;9(378):aah4680.
3. Williams MR, Costa SK, Zaramela LS, et al. Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis. *Sci Transl Med*. 2019;11(490):aat8329.
4. Paharik AE, Parlet CP, Chung N, et al. Coagulase-negative Staphylococcal strain prevents *Staphylococcus aureus* colonization and skin infection by blocking quorum sensing. *Cell Host Microbe*. 2017;22(6):746-756.
5. Holland DB, Bojar RA, Farrar MD, Holland KT. Differential innate immune responses of a living skin equivalent model colonized by *Staphylococcus epidermidis* or *Staphylococcus aureus*. *FEMS Microbiol Lett*. 2009;290(2):149-155.
6. Chng KR, Tay AS, Li C, et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nat Microbiol*. 2016;1(9):16106.
7. Rademacher F, Simanski M, Hesse B, et al. *Staphylococcus epidermidis* activates aryl hydrocarbon receptor signaling in human keratinocytes: implications for cutaneous defense. *J Innate Immun*. 2019;11(2):125-135.
8. Simanski M, Erkens AS, Rademacher F, Harder J. *Staphylococcus epidermidis*-induced Interleukin-1 beta and human beta-defensin-2 expression in human keratinocytes is regulated by the host molecule A20 (TNFAIP3). *Acta Derm Venereol*. 2019;99(2):181-187.
9. Hon KL, Tsang YC, Pong NH, Leung TF, Ip M. Exploring *Staphylococcus epidermidis* in atopic eczema: friend or foe? *Clin Exp Dermatol*. 2016;41(6):659-663.
10. Totte JEE, Pardo LM, Fieten KB, et al. Nasal and skin microbiomes are associated with disease severity in paediatric atopic dermatitis. *Br J Dermatol*. 2019;181(4):796-804.
11. Bottero V, Imbert V, Frelin C, Formento JL, Peyron JF. Monitoring NF-kappa B transactivation potential via real-time PCR quantification of I kappa B-alpha gene expression. *Mol Diagn*. 2003;7(3–4):187-194.
12. Cogen AL, Yamasaki K, Sanchez KM, et al. Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. *J Invest Dermatol*. 2010;130(1):192-200.
13. Iwase T, Uehara Y, Shinji H, et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature*. 2010;465(7296):346-349.
14. Liu Q, Liu Q, Meng H, et al. *Staphylococcus epidermidis* contributes to healthy maturation of the nasal microbiome by stimulating antimicrobial peptide production. *Cell Host Microbe*. 2020;27(1):68-78.
15. Ommori R, Ouji N, Mizuno F, Kita E, Ikada Y, Asada H. Selective induction of antimicrobial peptides from keratinocytes by staphylococcal bacteria. *Microb Pathog*. 2013;56:35-39.
16. Wanke I, Steffen H, Christ C, et al. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *J Invest Dermatol*. 2011;131(2):382-390.
17. Naik S, Bouladoux N, Wilhelm C, et al. Compartmentalized control of skin immunity by resident commensals. *Science*. 2012;337(6098):1115-1119.
18. Lai Y, Di Nardo A, Nakatsuji T, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med*. 2009;15(12):1377-1382.
19. Li D, Wang W, Wu Y, Ma X, Zhou W, Lai Y. Lipopeptide 78 from *Staphylococcus epidermidis* activates beta-catenin to inhibit skin inflammation. *J Immunol*. 2019;202(4):1219-1228.
20. Edslev SM, Agner T, Andersen PS. Skin microbiome in atopic dermatitis. *Acta Derm Venereol*. 2020;100(12):adv00164.
21. Bjerre RD, Holm JB, Palleja A, Solberg J, Skov L, Johansen JD. Skin dysbiosis in the microbiome in atopic dermatitis is site-specific and involves bacteria, fungus and virus. *BMC Microbiol*. 2021;21(1):256.
22. Kong HH, Oh J, Deming C, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res*. 2012;22(5):850-859.
23. Soares J, Lopes C, Tavaria F, Delgado L, Pintado M. A diversity profile from the staphylococcal community on atopic dermatitis skin: a molecular approach. *J Appl Microbiol*. 2013;115(6):1411-1419.
24. Cau L, Williams MR, Butcher AM, et al. *Staphylococcus epidermidis* protease EcpA can be a deleterious component of the skin microbiome in atopic dermatitis. *J Allergy Clin Immunol*. 2020;147(3):955-966.
25. Akiyama H, Kanzaki H, Tada J, Arata J. Coagulase-negative staphylococci isolated from various skin lesions. *J Dermatol*. 1998;25(9):563-568.
26. Otto M. *Staphylococcus epidermidis* pathogenesis. *Methods Mol Biol*. 2014;1106:17-31.
27. Liles WC, Thomsen AR, O'Mahony DS, Klebanoff SJ. Stimulation of human neutrophils and monocytes by staphylococcal phenol-soluble modulin. *J Leukoc Biol*. 2001;70(1):96-102.
28. Vuong C, Durr M, Carmody AB, Peschel A, Klebanoff SJ, Otto M. Regulated expression of pathogen-associated molecular pattern molecules in Staphylococcus epidermidis: quorum-sensing determines pro-inflammatory capacity and production of phenol-soluble modulins. *Cell Microbiol*. 2004;6(8):753-759.

29. Drislane C, Irvine AD. The role of filaggrin in atopic dermatitis and allergic disease. *Ann Allergy Asthma Immunol*. 2020;124(1):36-43.
30. Totsuka A, Omori-Miyake M, Kawashima M, Yagi J, Tsunemi Y. Expression of keratin 1, keratin 10, desmoglein 1 and desmocollin 1 in the epidermis: possible downregulation by interleukin-4 and interleukin-13 in atopic dermatitis. *Eur J Dermatol*. 2017;27(3):247-253.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1 Materials and Methods.

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