Crosstalk of Keratinocytes with Commensals and Neutrophils shapes *Staphylococcus aureus* Skin Colonization

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Katharina Bitschar
aus Friedrichshafen

Tübingen
2019

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	28.04.2020
Dekan:	Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter:	Prof. Dr. Birgit Schittek
2. Berichterstatter:	Prof. Dr. Andreas Peschel

Table of Contents

List of Figures

List of Abbreviations

AD	Atopic dermatitis
AIP	Autoinducing peptides
AMP	Antimicrobial peptide
CA	Community-associated
CCL	CC-chemokine ligand
CCR	CC-chemokine receptor
CD	Cluster of differentiation
CFU	Colony forming units
CHIPS	Chemotaxis inhibitory proteins
CI	Combination indices
citH3	Citrullinated histone H3
Clf	Clumping factor
CLR	C-type-lectin receptor
CM	Conditoned medium
CoNS	Coagulase-negative Staphylococci
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CXCL	C-X-C motif ligand
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
DCD	Dermcidin
DNA	Deoyribonucleic acid
ERK	Extracellular signal-regulated protein kinase
ERK	Extracellular signal-regulated protein kinase
FLIPr	Formyl peptide receptor-like 1 inhibitory
fMLF	N-formyl-met-leu-phe
FnBP	Fibronectin-binding protein
HA	Healthcare-associated
hBD	Human β-defensin
hCAP18	Human cathelicidin antimicrobial protein 18
HEK	Human embryonic kidney
HMGB1	High-Mobility-Group-Box 1

HNEpC	Primary human nasal epithelial cells
HTEpC	Primary human tracheal epithelial cells
IFNg	Interferonγ
Ig	Immunoglobuline
IL	Interleukin
ILC	Innate lymphoid cell
IRAK	Interleukin-1 receptor-associated kinase
IRF	Interferon regulatory factor
IsdA	Iron-regulated surface determinant A
LC	Langerhans cell
LPS	Lipopolysaccharide
LSM	Laser scanning microscopy
LTA	Lipoteichoic acid
LPP	Lipoprotein
MAPK	Mitogen-activated protein kinase
MIC	Minimal inhibitory concentration
MIP-2	Macrophage inflammatory protein 2
mo	Monocyte-derived
MOI	Multiplicity of infection
MPO	Myeloperoxidase
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant Staphylococcus aureus
MSCRAMM	Microbial surface components recognizing adhesive matrix molecule
MyD88	Myeloid differentiation primary response protein 88
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural Killer
NLR	NOD-like receptor
NOD	Nucleotide-binding oligomerization domain
Nuc	Staphylococcal nuclease
PAD4	Protein arginine deiminase 4
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline

PD-L1	Programmed cell death ligand 1
PHK	Primary human keratinocytes
PMA	Phorbol-myristate-actetat
PMN	Polymorphonuclear cells
PRR	Pattern recognition receptor
PSM	Phenol-soluble modulin
RIG	Retinoic acid-inducible gene
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
S.	*Staphylococcus*
SE	Staphylococcal enterotoxin
SSL3	Superantigen-like protein 3
STAT3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor 1
T reg	Regulatory T cell
TH	T helper
TIR	Toll/Interleukin-1 receptor
TLR	Toll-like receptor
TNFα	Tumor necrosis factor α
TRAF	Tumor necrosis factor receptor associated factor
TRIF	Toll-like receptor adaptor molecule 1
TSLP	Thymic stromal lymphopoietin
TSST-1	Toxic shock syndrome toxin 1
wt	Wild-type
WTA	Wall teichoic acid

1 Summary

Our skin is constantly exposed to a large number of pathogens while at the same time undergoing selective colonization by commensal microorganisms such as Coagulase-negative Staphylococci. *Staphylococcus aureus*, however, is a facultative pathogen that is usually absent from healthy skin but frequently colonizes the inflamed skin of atopic dermatitis (AD) patients where it further promotes inflammation. Thereby, increasing *S. aureus* colonization was shown to correlate with a loss of microbiome diversity indicating a role for skin commensals to shape pathogen colonization. Keratinocytes, as the most abundant and outermost cell type in the epidermis, need to discriminate commensals from pathogens and orchestrate subsequent immune reactions in response to colonizing microbes. However, the mechanisms how individual commensals cooperate with keratinocytes and the immune system of the skin to prevent pathogen colonization are barely understood.

Therefore, this work aimed at investigating the functional effects of two skin commensals, *S. epidermidis* and *S. lugdunensis,* on *S. aureus* skin colonization. Using an *in vitro* adhesion and invasion assay with primary human keratinocytes and an epicutaneous mouse skin colonization model we show that pretreatment with *S. epidermidis* or its secreted factors significantly reduces *S. aureus* skin colonization. However, in this work we also demonstrate that this protection is dependent on the integrity of the epithelial barrier and is completely lost during skin inflammation.

Using the same models, we further demonstrate that the *S. lugdunensis*-derived cyclic peptide antibiotic, lugdunin, which was previously shown to inhibit *S. aureus* epithelial colonization, induces a similar protective effect in human keratinocytes and mouse skin. Additionally, lugdunin can amplify the *S. epidermidis*-induced effect. Further analysis revealed that, beyond its bactericidal activity, lugdunin also possesses TLR/MyD88-dependent immune-modulatory activities which lead to expression and release of LL-37 and CXCL8/MIP-2 in human keratinocytes and mouse skin as well as to the recruitment of monocytes and neutrophils in mouse skin. Ultimately, synergistic antimicrobial activity in combination with skin-derived AMPs indicates that lugdunin is a multi-functional peptide providing host protection against *S. aureus* by multiple mechanisms.

Since *S. aureus* frequently colonizes the inflamed skin of AD patients, this work also aimed at understanding how inflammation contributes to the initial skin colonization event with *S. aureus*. We found that recruited neutrophils in response to tape-stripping enhance *S. aureus* colonization. These findings were confirmed using an *in vitro* co-culture model with keratinocytes and neutrophils. Further analysis of the mechanism demonstrated that neutrophil extracellular traps (NETs) influence keratinocytes in a way that favors *S. aureus* colonization. Finally, we show that *S. epidermidis* can reduce neutrophil recruitment induced by *S. aureus*, which might partly explain how microbiota contribute to pathogen skin protection during homeostasis. At the same time this work suggests that during skin inflammation the release of NETs by infiltrating neutrophils superimposes the microbiota-mediated skin protection and might thus result in enhanced *S. aureus* colonization.

In conclusion, this work describes how two members of our skin microbiota, *S. epidermidis* and *S. lugdunensis*, can prevent *S. aureus* skin colonization. Thus, it delineates new ways how commensals protect our skin from pathogens. In addition, the work presented here also illustrates that *S. epidermidis* contributes to increased *S. aureus* colonization during skin inflammation and that the recruitment of immune cells can have conflicting effects. Consequently, the signals of the skin microbiome need to be interpreted in the corresponding microenvironment.

2 Zusammenfassung

Unsere Haut ist einer Vielzahl von Krankheitserregern permanent ausgesetzt und wird gleichzeitig mit ausgewählten kommensalen Mikroorganismen wie den Coagulase-negativen Staphylokokken besiedelt. *Staphylococcus aureus* ist im Gegensatz dazu ein fakultativ-pathogener Erreger, der gesunde Haut normalerweise nicht kolonisiert. Auf entzündeter Haut von Atopikern ist er jedoch sehr häufig zu finden, wo er die Entzündung weiter stimuliert. Interessanterweise geht die erhöhte *S. aureus* Kolonisierung mit einem Verlust der Mikrobiom-Diversität einher. Dies deutet darauf hin, dass Kommensale die Hautkolonisierung von Krankheitserregern weitgehend beeinflussen. Keratinozyten, die vorwiegenden Zellen in der Epidermis, müssen in der Lage sein, Kommensale von Krankheitserregern zu unterscheiden um nachfolgende Immunreaktionen entsprechend zu koordinieren. Die Mechanismen, wie einzelne Kommensale zusammen mit Keratinozyten und dem Immunsystem die Haut vor Krankheitserregern schützen, sind jedoch bisher weitestgehend unerforscht.

Ein Ziel dieser Forschungsarbeit war es daher, die funktionellen Auswirkungen von zwei Kommensalen, *S. epidermidis* und *S. lugdunensis*, auf die *S. aureus* Kolonisierung der Haut zu untersuchen. Mit einem *in vitro* Adhäsions- und Invasionsassay mit primären humanen Keratinozyten und einem epikutanen Modell der Maushautkolonisierung zeigen wir, dass eine Vorbehandlung mit *S. epidermidis* oder mit dessen sekretierten Faktoren die *S. aureus* Kolonisierung signifikant reduziert. Wir stellen allerdings auch dar, dass dieser Schutz von der Integrität der Epithelbarriere abhängt und während einer Hautentzündung vollständig verloren geht.

Mit den gleichen Modellen zeigen wir außerdem, dass das zyklische Peptid-Antibiotikum Lugdunin, welches von *S. lugdunensis* produziert wird und für welches zuvor eine bakterizide Wirkung auf *S. aureus* gezeigt wurde, eine ähnliche Schutzwirkung in humanen Keratinozyten und in Maushaut hervorruft. Weitere Analysen ergaben, dass Lugdunin über seine bakterizide Wirkung hinaus, auch TLR/MyD88-abhängige immunmodulatorische Eigenschaften besitzt, die sowohl zur Expression und Freisetzung von LL-37 und CXCL8/MIP-2 in menschlichen Keratinozyten und in der Maushaut führen, als auch zur Rekrutierung von Monozyten und Neutrophilen in die Maushaut. Zusätzlich besitzt Lugdunin in Kombination mit

humanen antimikrobiellen Peptiden aus der Haut eine synergistische bakterizide Wirkung gegen *S. aureus*. Daraus ergibt sich, dass Lugdunin ein multifunktionales Peptid ist, das uns durch eine Vielzahl von Mechanismen vor *S. aureus* schützen kann.

Ein weiteres Ziel dieser Arbeit war es zu verstehen, wie eine Entzündung zur erstmaligen Besiedelung der Haut mit *S. aureus* beiträgt. Dabei fanden wir heraus, dass durch entzündungsauslösendes Tape-stripping Neutrophile rekrutiert werden, die die *S. aureus* Kolonisierung fördern. Diese Ergebnisse konnten mit einem *in vitro* Co-Kulturmodell mit Keratinozyten und Neutrophilen bestätigt werden. Weitere mechanistische Analysen zeigten, dass neutrophile extrazelluläre Fallen (NETs) im Co-Kultursystem freigesetzt werden und Keratinozyten so beeinflussen, dass die *S. aureus* Kolonisierung begünstigt wird. Interessanterweise konnte diese verstärkte Bildung von NETs *in vitro* als auch die Rekrutierung von Neutrophilen *in vivo* durch eine Vorbehandlung mit *S. epidermidis* reduziert werden. Dies erklärt zumindest teilweise, wie *S. epidermidis* zum Schutz vor *S. aureus* beiträgt. Gleichzeitig deutet diese Arbeit darauf hin, dass bei Hautentzündungen NETs von infiltrierenden Neutrophilen freigesetzt werden, wodurch es zu einer Überlagerung des durch *S. epidermidis* vermittelten Hautschutzes und somit zu einer erhöhten *S. aureus* Kolonisierung kommt.

Zusammenfassend zeigt diese Arbeit, wie zwei Bestandteile unserer Hautflora, *S. epidermidis* und *S. lugdunensis*, eine *S. aureus* Kolonisierung verhindern können und führt somit weitere Wege auf, wie Kommensale unsere Haut vor Pathogenen schützen. Allerdings wird auch dargestellt, dass *S. epidermidis* während einer Entzündung zur erhöhten *S. aureus* Kolonisierung beiträgt und dass die Rekrutierung von Immunzellen gegensätzliche Auswirkungen haben kann. Folglich müssen die Signale des Hautmikrobioms in Abhängigkeit der entsprechenden Mikroumgebung interpretiert werden.

3 List of Publications

3.1 Accepted Publication I

Burian M., Bitschar K., Dylus B., Peschel A. and Schittek B. The Protective Effect of Microbiota on *S. aureus* Skin Colonization Depends on the Integrity of the Epithelial Barrier. J Invest Dermatol. 2017, April; 137(4):976-979; doi: 10.1016/j.jid.2016.11.024.

3.2 Accepted Publication II

Bitschar K., Sauer B., Focken J., Dehmer H., Moos S., Konnerth M., Schilling N.A., Grond S., Kalbacher H., Kurschus F.C., Götz F., Krismer B., Peschel A. and Schittek B. Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. Nat Commun. 2019 Jun 21;10(1):2730. doi: 10.1038/s41467-019-10646-7.

3.3 Accepted Publication III

Bitschar K., Wolz C., Krismer B., Peschel A. and Schittek B. Keratinocytes as sensors and central players in the immune defense against Staphylococcus aureus in the skin. J Dermatol Sci. 2017 Sep;87(3):215-220. doi: 10.1016/j.jdermsci.2017.06.003.

3.4 Accepted Manuscript I

Bitschar K., Staudenmaier L., Focken J., Klink L., Sauer B., Fehrenbacher B., Herster F., Bittner Z., Bleul L., Schaller M., Wolz C., Weber A. N. R., Peschel A. and Schittek B. Staphylococcus aureus skin colonization is enhanced by the interaction of neutrophil extracellular traps with keratinocytes.

4 Personal Contributions

4.1 Accepted Publication I

- I performed 50% of the adhesion and invasion assays (Figure 1b+c) and 50% of the mouse experiments (Figure 2b-e) and analyzed the respective data.
- I designed Figure 1b-e and Figure 2b-e.
- I helped in proof-reading the manuscript.

4.2 Accepted Publication II

- Together with B. Schittek, I concepted, designed and supervised this study and developed all necessary methodologies.
- I performed all experiments with assistance of J. Focken (qRT-PCR, AMT assays), B. Sauer (immunohistochemistry) and H. Dehmer (AMT assays).
- I designed all experiments and figures.
- I analyzed the data and wrote the manuscript under the guidance of B. Schittek.
- S. Moos (immune cell isolation) and H. Dehmer (AMT assays) further contributed to the development of methodology.
- B. Krismer and A. Peschel further helped in proof-reading the manuscript.
- Lugdunin was provided by Martin Konnerth (natural lugdunin), N. Schilling (synthetic lugdunin).
- All other co-authors (S. Moos, S. Grond, H. Kalbacher, F.C. Kurschus, F. Götz, B. Krismer, A. Peschel) contributed to this publication by administrative, technical or material support.

4.3 Accepted Publication III

- Together with B. Schittek and C. Wolz I wrote the manuscript.
- Together with B. Schittek I designed the figure.
- B. Krismer and A. Peschel further contributed by proof-reading the manuscript.

4.4 Accepted Manuscript I

- Together with B. Schittek, I concepted, designed and supervised this study and developed all necessary methodologies.
- I performed all experiments with assistance of L. Klink (co-culture experiments), J. Focken (co-culture experiments, mouse immune cell isolation) and B. Sauer (immunohistochemical staining) for the first submission. Required experiments (dsDNA ELISA, genomic DNA isolation, PAD4 inhibition) for the revision of the manuscript were performed by L. Staudenmaier.
- I designed all experiments except for dsDNA ELISA, genomic DNA isolation, PAD4 inhibition which were designed by L. Staudenmaier.
- I designed all figures.
- I analyzed the data and wrote the manuscript under the guidance of B. Schittek.
- L. Klink (co-culture), Z. Bittner (neutrophil isolation) and F. Herster (neutrophil isolation) further contributed to the development of methodology.
- B. Schittek and A. Weber further helped in proof-reading the manuscript.
- All other co-authors (B. Fehrenbacher, F. Herster, Z. Bittner, L. Bleul, M. Schaller, C. Wolz, A. N. R. Weber, A. Peschel) contributed to this publication by administrative, technical or material support.

5 Introduction

5.1 Skin immunity

The primary function of our skin is to serve as a physical barrier that protects our body from environmental insults while at the same time it prevents water loss. From the moment of birth, our skin, as the outermost layer to the environment, is also constantly exposed to a large set of different microbes including potential pathogens. Therefore, it must be actively involved in immune defense that allows commensal microbes to colonize while at the same time pathogenic bacteria such as *Staphylococcus aureus* are effectively prevented from colonizing. This is achieved mainly by the keratinocytes, the major cell type of the epidermis, which sense colonizing microbes and coordinate subsequent immune responses in the skin. Expression of antimicrobial peptides (AMPs) by keratinocytes provides a first level of defense against invading pathogens. Moreover, using different pattern recognition receptors (PRRs) keratinocytes can detect and discriminate commensals from pathogens and initiate an adequate response by secreting cytokines and chemokines which subsequently recruit and activate immune cells (Accepted Manuscript I (Bitschar et al., 2017)). In addition to keratinocytes, different immune cell subsets reside throughout the skin and contribute to skin homeostasis and immunity (Pasparakis et al., 2014).

Structurally the skin is composed of two distinct layers: the epidermis and the dermis (Figure 1). The epidermis forms the outermost barrier to the environment and is composed of layers of differentiated keratinocytes. The basal layer is the stratum basale which mainly consists of undifferentiated, dividing keratinocytes, that are constantly renewing the epidermis. Within 4 weeks of differentiation, keratinocytes move towards the outer layers, first forming the stratum spinosum followed by the stratum granulosum and finally the stratum corneum building the outermost layer which consists of terminally differentiated, dead and cornified keratinocytes that are chemically crosslinked and continuously shed from the skin surface (Fuchs and Raghavan, 2002; Segre, 2006). These cornified cells are surrounded by lipids which provide a physical and water-retaining barrier of the skin (Candi et al., 2005). In addition to keratinocytes, melanocytes and Langerhans cells are dispersed

throughout the epidermis. While the former contribute to UV radiation protection, the latter are antigen-presenting cells involved in cutaneous immune defense.

Below the epidermis, the dermis harbors fibroblasts which produce extracellular matrix proteins such as collagen and elastin in order to provide structural support for the skin. Additionally, the dermis anchors blood and lymphatic vessels as well as different appendages such as sweat and sebaceous glands or hair follicles. It also harbors a multitude of different resident as well as patrolling immune cells that contribute to tissue homeostasis and skin immunity. Underneath the dermis lies the hypodermis which constitutes an important depot of fat and is involved in thermoregulation (Brown and Krishnamurthy, 2019).

In the following paragraph, the importance of an intact skin barrier with functional skin immunity is exemplified by the prevalence of inflammatory skin disorders such as atopic dermatitis and psoriasis, both of which are chronic skin diseases characterized by keratinocytes that respond to opposing aberrant T cell signaling. Subsequently, the general components and mechanisms of skin immunity under steady-state, during staphylococcal skin colonization and in the context of inflammatory skin diseases are illustrated.

5.1.1 Inflammatory skin disorders: atopic dermatitis and psoriasis

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disorder that affects 5-20% of infants with higher prevalence in industrialized countries most likely due to increased hygienic conditions and concomitant lack of microbial education of the immune system (Deckers et al., 2012; Ring et al., 2001; Williams et al., 2008). AD is a complex heterogeneous disease with many different factors potentially contributing to its development and pathogenesis such as genetic predisposition, environmental impacts, skin barrier impairment, T helper type 2 (T_H2) cell activation and dysbiosis of the skin microbiome. Genome-wide scans have revealed that there are many shared chromosome loci among AD patients clearly demonstrating how complex this disease is (Bieber, 2008; Eyerich et al., 2015; Weidinger et al., 2018). For example, 10% of AD patients are known to have a mutation in the filaggrin (*FLG*) gene, a major component of the stratum corneum. Consequently, this mutation results in increased trans-epidermal water loss and skin barrier impairment (Irvine et al., 2011). In response to a defective skin barrier, keratinocyte-mediated secretion of

cytokines such as thymic stromal lymphopoietin (TSLP), Interleukin (IL)-25 and IL-33 have emerged as potent key drivers of T_H2 inflammation which is considered a hallmark of AD (Han et al., 2017). In addition, prolonged antigen penetration through the defective barrier may stimulate the observed T_H2 infiltrate. It has been reported that T_H2 and T_H22 cytokines such as IL-4, IL-13 and IL-22 are increased in the lesional skin of AD patients (Gittler et al., 2012) which in turn lead to the downregulation of skin barrier proteins such as filaggrin, loricrin and involucrin by keratinocytes (Cornelissen et al., 2012; Howell et al., 2009) thus starting a vicious cycle. In line with this, the monoclonal antibody dupilumab, which inhibits Th2 signaling by blocking the IL-4 receptor has been shown to ameliorate AD (Beck et al., 2014).

In addition to T_H2 cytokines, a T_H2/T_H1 switch has been shown to contribute to the chronic development of AD while T_H22 and T_H17 cytokines were shown to contribute to the initiation of AD (Czarnowicki et al., 2015; Gittler et al., 2012). Moreover, there is increasing evidence that dysbiosis of the microbiome contributes to AD development. Opposed to healthy skin, *S. aureus* is highly prevalent on AD skin and therefore drives skin inflammation even further. The reasons for increased *S. aureus* colonization have remained elusive, however, it is likely that altered nutrient availability due to barrier impairment as well as the resulting dysbiosis of the microbiome contribute to increased *S. aureus* colonization on AD skin (Kong et al., 2012). The role of S. aureus in AD is further described in section 5.2.3.2.

Psoriasis is a chronic inflammatory skin disease characterized by infiltration of T_H17 cells and neutrophils as well as by hyperproliferation of keratinocytes. During psoriasis pathogenesis early danger signals or skin trauma induce infiltration of T_H17 cells into the skin where they secrete T_H17 cytokines such as IL-17A/F, IL-21, and IL-22. Keratinocytes do not express IL-17A/F cytokines but their receptor components IL-17RA and IL-17RC (Liang et al., 2006). Binding of IL-17A/F to the IL-17 receptor results in the expression of AMPs and neutrophil-attracting chemokines such as CXCL8 leading to neutrophil recruitment to the epidermis and initiation of a pro-inflammatory loop (Albanesi et al., 2007). In addition, IL-17 cytokines induce aberrant differentiation of keratinocytes resulting in hyperproliferation and thickening of the epidermis (Albanesi et al., 2007). In a psoriasis mouse model, it was recently shown that IL-17 signaling in keratinocytes critically contributes to the development of psoriasis and that depletion of the IL-17 receptor in keratinocytes but not in T cells,

neutrophils or macrophages, protects mice from psoriasis development (Moos et al., 2019). Moreover, neutralizing therapies against IL-17A are highly efficient in the treatment of psoriasis patients (Kurschus and Moos, 2017). Opposed to AD, increased levels of IL-17 cytokines and concomitant high levels of AMPs prevent increased colonization with *S. aureus*, however, a role for microbiome dysbiosis has also been implicated in the pathogenesis of psoriasis (Visser et al., 2019).

5.1.2 Antimicrobial peptides of the skin

AMPs are an evolutionarily conserved component of the innate immune system. They are multi-functional host defense peptides with broad spectrum antimicrobial activity against a wide range of pathogens including bacteria, fungi, and viruses. Additionally, AMPs display other features such as wound healing capacities and immuno-modulatory activities and thus provide a link from innate to adaptive immunity (Bitschar et al., 2017; Niyonsaba et al., 2017; Niyonsaba et al., 2009a; Schittek, 2011). While some AMPs are constitutively expressed, expression of others can be induced upon injury or infection (Schittek, 2011). The main cellular sources of AMPs in the skin are the keratinocytes but also other skin cells or immune cells express AMPs (Niyonsaba et al., 2009a). The major AMP families comprise defensins, cathelicidins, dermcidin, S100 proteins and ribonucleases and until today above 3000 AMPs have been reported in the AMP database (http://aps.unmc.edu/AP/main.php; accessed April 2019):

Defensins are short cationic peptides which exhibit their antimicrobial activity by forming pores in the negatively charged microbial membranes resulting in cell death (Niyonsaba et al., 2009a). In contrast to α-defensins, which are mainly expressed by neutrophils, β-defensins (hBDs) are predominantly expressed in epithelial cells (Niyonsaba et al., 2009a). Generally, hBD1 is considered being constitutively expressed in keratinocytes, whereas expression of hBD2, hBD3 and hBD4 can be induced in response to bacterial skin infection and pro-inflammatory stimuli. It was shown that hBD3 expression in human keratinocytes effectively leads to *S. aureus* killing within minutes of contact with the cell surface (Kisich et al., 2008). However, in patients suffering from AD, T_H2 cytokines were shown to inhibit the expression of hBD3 and also block its antimicrobial activity on *S. aureus* (Kisich et al., 2008). Nevertheless, a stronger induction of hBD3 expression is associated with a more favourable clinical outcome of *S. aureus* skin infections (Zanger et al., 2010).

Additionally, it was shown that high baseline expression levels of RNase7 provide protection against *S. aureus* skin infection, while there was no association with hBD2 or hBD3 expression, suggesting that hBD3 is involved in containment of *S. aureus* infection while RNAse7 expression contributes to infectious susceptibility (Simanski et al., 2010; Zanger et al., 2010).

The only human **cathelicidin** LL-37, with its precursor human cathelicidin antimicrobial protein 18 (hCAP18), is mainly expressed in neutrophils but expression can also be induced in keratinocytes upon stimulation with pathogenic bacteria or pro-inflammatory stimuli (Niyonsaba et al., 2009a; Schroder and Harder, 2006). By inducing pore formation and concomitant membrane disruption, the cationic peptide LL-37 exhibits bactericidal and anti-biofilm properties against *S. aureus* (Dean et al., 2011; Overhage et al., 2008; Zaiou et al., 2003). Additionally, LL-37 was shown to promote wound healing (Carretero et al., 2008) and contributes to homeostasis of the epidermal skin barrier (Akiyama et al., 2014). However, in psoriatic skin excessive production of AMPs, especially LL-37, was shown to contribute to disease pathogenesis (Furue and Kadono, 2019).

Dermcidin and its proteolytically processed active peptides DCD-1 and DCD-1L, on the other hand, are constitutively expressed in eccrine sweat glands where they can be transported to the skin surface and serve as an antimicrobial shield (Rieg et al., 2004; Schittek et al., 2001). Dermcidin peptides exert a different mode of action than most other AMPs: owing to its anionic surface charge dermcidin can interact with positively charged Zink ions leading to pore-formation in the bacterial membrane and concomitant killing (Becucci et al., 2014; Paulmann et al., 2012). Interestingly, AD patients were shown to exhibit a reduced level of dermcidin-derived peptides in their sweat which correlated with an impaired ability of their sweat to eradicate viable bacteria compared to sweat from healthy people (Rieg et al., 2005; Schittek, 2011).

When compared to psoriatic skin expression of AMPs was shown to be generally reduced in AD (Nomura et al., 2003; Ong et al., 2002), this reduction does not explain the increased *S. aureus* colonization in AD patients because AMP expression was still higher than in healthy skin (Harder et al., 2010).

5.1.3 Immune cells of the skin

Many different subsets of innate and adaptive immune cells either reside in or can be recruited to the skin. These cell populations, however, are highly dynamic and can undergo dramatic changes upon skin infection (Pasparakis et al., 2014). In the following paragraphs and in Figure 1 the major innate and adaptive immune cell subsets of the skin are described:

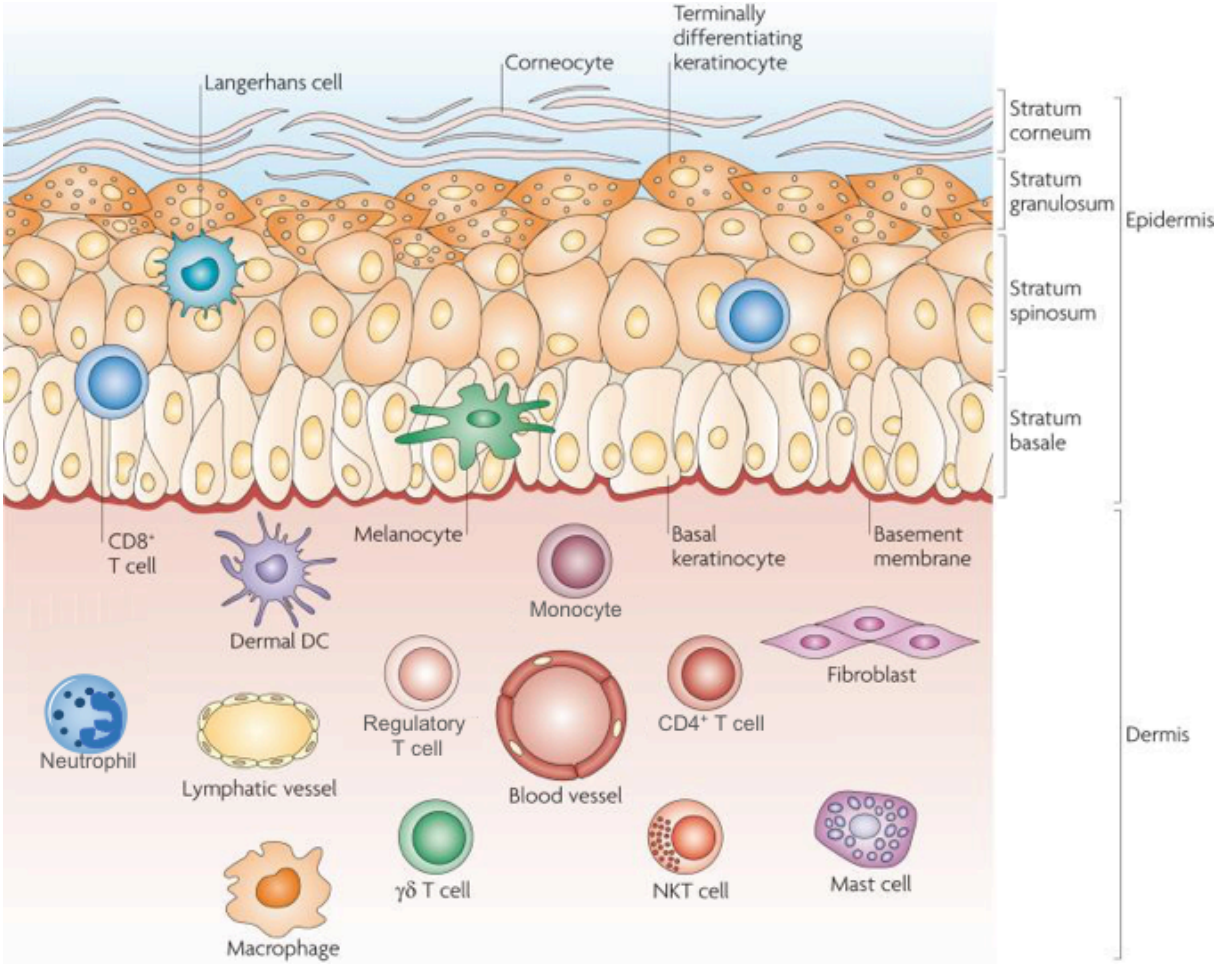


Figure 1: Skin structure and immune cells of the skin. Adapted from (Nestle et al., 2009)

The skin is composed of two layers: the epidermis and the dermis. The epidermis is composed of layers of differentiated keratinocytes. The basal layer is the stratum basale which mainly consists of undifferentiated keratinocytes, that are constantly renewing the epidermis. During differentiation, keratinocytes move towards the outer layers, forming the stratum spinosum, stratum granulosum and finally the stratum corneum which consists of terminally differentiated, dead and cornified keratinocytes. In addition to keratinocytes, melanocytes and Langerhans cells as well as $CD8^+$ T cells are dispersed throughout the epidermis. In addition to fibroblasts and blood and lymphatic vessels, the dermis harbors a multitude of different immune cells that contribute to tissue homeostasis and skin immunity. While some immune cells can be tissue-resident (such as macrophages or dermal dendritic cells) others are patrolling or can be recruited upon tissue damage or infection (such as monocytes or neutrophils).

5.1.3.1 Keratinocytes

In addition to providing a physical barrier to the environment, keratinocytes as the main constituents of the epidermis are responsible for initiation of the magnitude as well as the type of local immune responses. During their maturation process keratinocytes move from the basal to the uppermost layer of the epidermis where they are strategically positioned between the body and the environment to sense microbes and thus can subsequently orchestrate adequate immune responses (Fuchs and Raghavan, 2002; Segre, 2006). However, keratinocytes are often underestimated as part of the innate immune system. Communication of environmental signals can be translated by keratinocytes to the immune system by expression of different PRRs (see section 5.1.4), activation of which result in distinct intrinsic signaling pathways such as mitogen-activated protein (MAP) kinase or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling which in turn lead to the production of different pro-inflammatory cytokines and chemokines (SanMiguel and Grice, 2015). Additionally, expression of different cytokine and chemokine receptors allows keratinocytes to respond to immunologic signals (section 5.1.5). For example, expression of the IL-17A receptor results in AMP production by keratinocytes in response to T cells (Liang et al., 2006). The role of keratinocytes as sensors and central players in the immune defense against *S. aureus* is further described in Accepted Publication III (Bitschar et al., 2017).

5.1.3.2 Myeloid cells

Neutrophils are the most abundant leukocytes of the human blood and the first line of defense once pathogens have breached the epithelial barrier. They are usually not present in healthy skin but chemokines and cytokines secreted by keratinocytes can recruit them along chemotactic gradients to the site of infection in order to clear pathogens (de Oliveira et al., 2016; Kolaczkowska and Kubes, 2013). Pathogen killing by neutrophils mainly occurs via phagocytosis and subsequent fusion of different types of neutrophilic granules with the phagosome. Primary, azurophil granules are a specialized form of the lysosome and contain many proteins that are directly involved in microbial killing and digestion such as AMPs, myeloperoxidase (MPO) or proteinases such as cathepsin G, elastase or proteinase 3 (Segal, 2005). Secondary granules mainly contain lactoferrin to bind and sequester iron and copper in order to prevent the formation of highly reactive hydroxyl radicals (Britigan et al.,

1989). Gelatinase, which allows the neutrophil to penetrate endothelial basement membranes, is present both in the secondary granules as well as in the tertiary granules which, however, do not contain lactoferrin (Segal, 2005). Upon phagocytosis, neutrophils intracellularly expose the pathogen to AMPs and reactive oxygen species (ROS) followed by an apoptotic differentiation program that limits host damage caused by a prolonged inflammatory response (Kennedy and DeLeo, 2009; Kobayashi et al., 2017). Subsequent clearance of apoptotic neutrophils by macrophages, a process referred to as efferocytosis, further prevents excessive inflammation and tissue damage (Martin et al., 2012). *S. aureus,* however, was shown to counteract this process by the production of the pore-forming α-toxin which lyses neutrophils and by this promotes necroptosis and a concomitant inflammatory response (Cohen et al., 2016; Greenlee-Wacker, 2016; Greenlee-Wacker et al., 2014). Apart from inhibition of macrophage efferocytosis, *S. aureus* has evolved multiple other strategies to evade neutrophil-mediated phagocytosis (Guerra et al., 2017). These strategies are further described in section 5.2.3 as well as in Accepted Publication III.

The discovery of neutrophil extracellular traps (NETs) significantly contributed to the repertoire of weapons employed by neutrophils to kill bacteria (Brinkmann et al., 2004): NETs are large extracellular, web-like structures composed of granule proteins and AMPs that are assembled on decondensed chromatin and are extruded from neutrophils in order to trap and kill microbes (Brinkmann et al., 2004). The composition of NET proteins as well as the pathways that lead to NET formation were shown to vary depending on the stimulus (Boeltz et al., 2019; Kenny et al., 2017). Figure 2 schematically illustrates the formation of NETs by neutrophils. Generally, neutrophils first arrest their actin dynamics and depolarize (Metzler et al., 2014), followed by disassembly of the nuclear envelope and decondensation of nuclear chromatin into the cytoplasm where it mixes with cytoplasmic and granule components (Fuchs et al., 2007). Here, neutrophil elastase and MPO binding of chromatin was shown to synergistically drive chromatin unpacking (Papayannopoulos et al., 2010). Moreover, global activation of transcription at multiple loci as well as hypercitrullination (Wang et al., 2009) and proteolytic degradation (Urban et al., 2009) of histones was shown to further promote chromatin decondensation (Khan and Palaniyar, 2017). Finally, the plasma membrane permeabilizes, and NETs expand into the extracellular space 3-8 hours after neutrophil activation (Papayannopoulos,

2018). Additionally, alternative mechanisms that describe non-suicidal pathways of NET formation are described in the literature: For example, exposure to *S. aureus* was shown to lead to rapid, non-lytic secretion of chromatin and granules (Pilsczek et al., 2010). Yipp et al. (2012) further describe the occurrence of anuclear "neutrophil ghosts", referring to a state after NET formation where cellular functions such as chemotaxis and phagocytosis remain intact (Yipp et al., 2012).

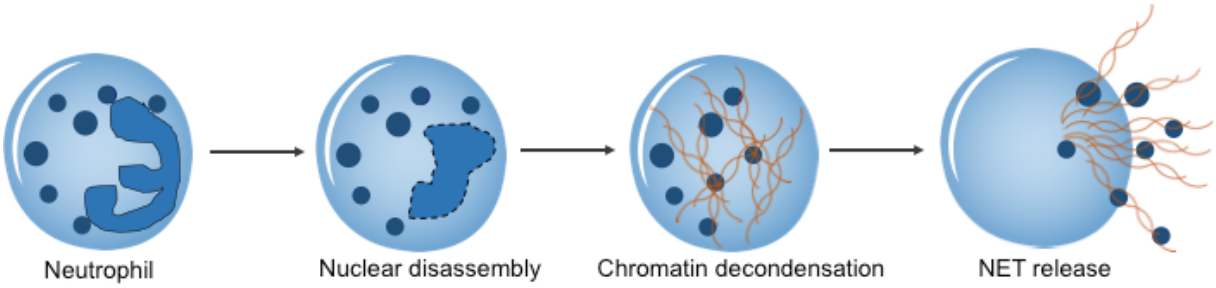


Figure 2: Schematic representation of neutrophil extracellular trap formation

Neutrophils typically possess a multi-lobed nucleus (blue) and several granules (dark blue). In order to form NETs, neutrophils first arrest their actin dynamics followed by disassembly of the nuclear envelope (dashed line) and decondensation of nuclear chromatin (orange). In the cytoplasm nuclear chromatin mixes with cytoplasmic and granule components such as neutrophil elastase and myeloperoxidase which further drives chromatin unpacking. Finally, the plasma membrane permeabilizes and NETs expand into the extracellular space.

Dendritic cells (DCs) in humans and mice are classified as plasmacytoid and conventional DCs. In peripheral tissues, conventional DCs can be further categorized into tissue-resident and migratory DCs (Merad et al., 2013). Specialized skin-resident DCs are the **Langerhans cells** (LCs) which are representatives of the migratory DCs and colonize the epidermis as well as other epithelia such as the gastrointestinal or bronchial tracts (Sparber, 2014). LCs arise from hematopoietic precursors and seed the epidermis before birth. During the first week after birth LCs proliferate and create a dense network in the epidermis where they constitute approximately 3% of all cells (Chorro et al., 2009). Thereby, a paracrine supply of TGF-β provided by keratinocytes is needed for the development and survival of LCs (Kaplan et al., 2007). Their dendritic morphology extends through tight junctions to the stratum corneum where LCs capture microbial antigens (Nagao et al., 2009). LCs express a wide variety of PRRs including surface C-type lectins such as langerin (CD207) (Valladeau et al., 2000) or CD1a (Salamero et al., 2001). These PRRs recognize mannosylated ligands found on the surface of a wide range of microbes (Dambuza and Brown, 2015; Figdor et al., 2002) and induce antigen internalization and

trafficking to the Birbeck granules where antigens are processed (Stossel et al., 1990; Valladeau et al., 2000). Upon antigen recognition and processing LCs upregulate MHC class II and concurrently migrate to regional lymph nodes where antigens are presented to naïve T cells (Koch et al., 2006). Thereby, the presence of distinct LC-secreted cytokines determines the type of induced immune response. It appears that LCs are capable of inducing both tolerance as well as immunity (Atmatzidis et al., 2017): For example, in mice LCs were shown to promote the induction of Th17 cells in response to extracellular pathogens, while it has also been observed that LC-mediated antigen-presentation results in the depletion of T cells suggesting an immuno-regulatory role (Igyarto et al., 2011; Igyarto and Kaplan, 2013). Compensation for emigrated LCs is carried out both by adjacent proliferative LCs (Ghigo et al., 2013) as well as replacement of epidermal LCs by monocytes (Nagao et al., 2012; Sere et al., 2012). LC maintenance and repopulation were shown to depend on keratinocytes signaling via colony stimulating factor 1 receptor (CSF1R), Macrophage colony stimulating factor (M-CSF), IL-1, tumor necrosis factor α (TNFα), CC-chemokine ligand 2 (CCL2) and CCL20 (Kimber et al., 2000; Nagao et al., 2012).

In addition to LCs, multiple different subsets of dermal DCs were identified in human and murine skin and were shown to contribute to cutaneous immunity both during skin homeostasis and skin infection (Tomura et al., 2014). For example, in murine skin *S. epidermidis*-induced accumulation of $CD8^+$ T cells was shown to be dependent on a subset of $CD103^+$ dendritic cells (Naik et al., 2015).

In addition to keratinocytes and LCs, resident skin **macrophages** are early detectors of pathogens entering the body through the skin and can initiate an inflammatory host response (Pasparakis et al., 2014). They are specialized in phagocytosis and neutralization of cellular debris and potentially host cell damaging agents including pathogens. In contrast to LCs, which trigger remote T cell responses upon translocation to tissue-draining lymph nodes, tissue-resident macrophages are non-migratory cells that locally monitor their immediate and adjacent surroundings (Mildner and Jung, 2014; Varol et al., 2015). Similarly to LCs, the majority of tissue-resident macrophages is established prenatally but macrophages can also develop in adulthood from tissue-infiltrating monocytes (Varol et al., 2015). Based on their function macrophages can generally be divided into three groups: The classically-activated pro-inflammatory M1 macrophages, regulatory M2 macrophages and

wound-healing macrophages. There is accumulating evidence that macrophages contribute to both acute and chronic inflammation in the skin: For example, pro-inflammatory M1 macrophages were abundantly observed in psoriatic skin (Fuentes-Duculan et al., 2010; Wang et al., 2019) as well as in skin lesions of AD patients (Kiekens et al., 2001). By contrast, regulatory M2 macrophages secrete IL-10 and TGFβ and thus have an essential role in resolution and dampening of skin inflammation (Mantovani et al., 2013). In *S. aureus* skin infection, upregulation of phagocytosis in M2 macrophages significantly contributed to infection clearance and prevention of excessive inflammation by limiting the number of available pathogens (Chiang et al., 2012).

Mast cells are hematopoietic cells that circulate in the blood in an immature state. Depending on the stimulus mast cells migrate to peripheral tissues and mature into multi-faceted effector cells which can exert a variety of different functions involved in immunity. They can activate and recruit other effector cells, directly neutralize microbes and are also involved in tissue remodeling and angiogenesis (Dudeck et al., 2018). In the skin, maturation of mast cells was shown to be dependent on microbiome-induced TLR2-mediated secretion of stem cell factor by keratinocytes (Wang et al., 2017). Within their cytoplasmic granules, large amounts of pre-formed, pro-inflammatory mediators are stored which can be rapidly released upon pathogen recognition in order to guarantee fast host protection. TLR2-mediated sensing of *S. aureus* was shown to result in rapid degranulation and discharge of AMPs and TNFα resulting in direct killing of *S. aureus* as well as in recruitment of other effector cells (Abel et al., 2011). However, mast cell-mediated release of IgE was also shown to contribute to AD pathogenesis. AD patients are frequently colonized with *S. aureus* and IgE antibodies directed against staphylococcal antigens are commonly found in their blood (Bunikowski et al., 1999; Ide et al., 2004; Leung et al., 1993). In an epicutaneous mouse skin colonization model staphylococcal δ-toxin was identified to be responsible for enhanced IgE and IL-4 production and concomitant inflammation enhancement. Additionally, it was shown that *S. aureus* strains isolated from AD patients produce large amounts of δ-toxin (Nakamura et al., 2013). Moreover, lesional skin of AD patients harbor high amounts of mast cells (Liu et al., 2011) and since *S. aureus* was also shown to survive and hide within mast cells, these cells might also serve as a reservoir of viable bacteria in AD skin (Nakamura et al., 2013).

5.1.3.3 Lymphoid cells

The **innate lymphoid cell** (ILC) family comprises lymphoid tissue-inducer cells, cytotoxic natural killer (NK) cells and non-cytotoxic ILCs. Similar to $CD8^+$ cytotoxic T cells, cytotoxic NK cells are involved in killing infected and tumor cells, while non-cytotoxic ILCs are the innate counterparts to effector T cells. ILCs can be further sub-grouped into ILC1,2 and 3. Analog to their effector T cell subsets, the ILC subgroups are defined based on their required transcription factors during development and their cytokine expression patterns. While ILC1s are implicated in immunity against intracellular pathogens, ILC2s produce T_H2 cytokines and thus promote type 2 inflammation in response to parasites and allergens. Consequently, ILC3s produce T_H17 cytokines and promote antibacterial and antifungal immunity. However, unlike their effector T cell counterparts, ILCs lack expression of somatically rearranged antigen receptors and thus act in an antigen-independent manner (Artis and Spits, 2015; Vivier et al., 2018; Yang et al., 2017). While NK cells circulate in the blood, ILCs are tissue-resident cells that are preferentially enriched in barrier tissues such as the skin, where they can instantly react to cytokines and other signals expressed by surrounding cells and participate in local host defense, tissue homeostasis and inflammation (Artis and Spits, 2015; Vivier et al., 2018; Yang et al., 2017). In lesional skin of AD patients elevated levels of T_H2 cytokine-producing ILC2s have been reported (Kim et al., 2013; Salimi et al., 2013). Moreover, in a mouse model of AD ILC2s were critically involved in the development of AD-like skin inflammation. Strikingly, ILC2-mediated AD-like inflammation was dependent on TSLP expression in mouse skin (Kim et al., 2013). In conclusion, due to their T_H2 analogy ILC2s might also contribute to the pathology of AD.

In addition to innate immune cells, representatives of adaptive immunity are largely present in the skin. It is estimated that $2*10^{10}$ **T cells** reside in healthy human skin which equals twice the number of T cells in the blood (Clark et al., 2006). Following microbial encounters, LCs migrate from the skin to the draining lymph nodes where microbial antigens are presented to naïve T cells (Koch et al., 2006). This results in the generation of effector and memory T cells that travel to the site of infection (Edele et al., 2008; Mora et al., 2005). Thereby the cytokine profile of the LC determines whether T cells undergo T_H1, T_H2 or T_H17 polarization (Atmatzidis et al., 2017). Effector T cells eradicate the infection and following infection resolution the majority of effector T cells dies while a small number becomes memory T cells which

either recirculate or reside in the skin where they are strategically positioned to provide a first response upon re-encounter with skin pathogens (Mueller et al., 2013).

In contrast to human skin, which is mainly populated by $CD4^+$ or $CD8^+$ $T_{\alpha\beta}$ cells, murine skin is densely populated with $T_{\gamma\delta}$ cells which seed the skin before birth (Jiang et al., 2010). Unlike αβT cells, $T_{\gamma\delta}$ cells do not recognize peptides bound to MHC molecules and until today the antigens recognized by $T_{\gamma\delta}$ cells remain largely unknown but are believed to play a role in microbial recognition (Adams et al., 2015). Indeed, it was shown that commensals drive the expansion of $T_{\gamma\delta}$ cells at barrier sites in an IL-1 and IL-23-dependent manner (Duan et al., 2010; Naik et al., 2012). In addition, $T_{\gamma\delta}$ cells can also be directly activated by cytokines such as IL-1 cytokines and IL-23 (Gray et al., 2011).

The magnitude of an immune response is effectively controlled by regulatory T cells (T_{regs}) which comprise 5–10% of all skin-resident T cells (Clark et al., 2006). In addition to that, most blood-circulating T_{regs} express skin-homing markers suggesting constitutive trafficking and patrolling to the skin (Hirahara et al., 2006). T_{regs} are key regulators of immune tolerance and block excessive inflammatory responses by the production of inhibitory cytokines, cytolysis of effector cells, metabolic disruption or modulation of DC function (Sakaguchi, 2005). In addition, T_{regs} were shown to facilitate cutaneous wound healing (Nosbaum et al., 2016). Interestingly, a large proportion of T_{regs} can be found in close proximity to the hair follicles which serve as a natural habitat for the microbiota (Sanchez-Rodriguez et al. 2014). Here, T_{regs} regulate the proliferation and differentiation of hair follicle stem cells (Ali et al., 2017). Moreover, during hair follicle development and concomitant commensal colonization, murine skin is populated by a wave of T_{regs} that provides tolerance to commensals also in the context of skin inflammation (Scharschmidt et al., 2017; Scharschmidt et al., 2015).

Although T cells coordinate cutaneous immune responses against microorganisms and cancer, aberrant and inappropriate T cell activation can lead to chronic inflammatory disorders such as AD or psoriasis. Interestingly, AD and psoriasis where shown to have opposing T cell cytokine profiles: While AD is dominated by infiltrates with T_H2 cells, psoriasis is driven by T_H17 cells (Eyerich et al., 2011).

5.1.4 Pattern recognition receptors

Keratinocytes and other cells of the innate immune system express several PRRs which are located on the cell surface, in endosomes or in the cytoplasm and detect pathogen-associated molecular patterns (PAMPs) or endogenous danger-associated molecular patterns (DAMPs) (Akira et al., 2001; Janeway and Medzhitov, 2002). Activation of PRRs in keratinocytes results in the production of AMPs and pro-inflammatory cytokines and chemokines by which subsequent adequate immune responses are induced. The innate immune system comprises several classes of PRRs which allow the early detection of potential pathogens: Toll-like receptors (TLRs), C-type-lectin receptors (CLRs), NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs) (Perez-Lopez et al., 2016). The membrane bound TLRs and CLRs detect PAMPs in the extracellular milieu and in the endosomal compartment. TLRs and CLRs can also cooperate with other PRRs such as RNA-sensing RIG-I-like receptors or DNA-sensing NLRs. For example, in the presence of TLR signals *S. aureus*-induced NOD2 activation amplified an inflammatory response in DCs (Volz et al., 2010). Upon cellular stress such as infection or metabolic deregulation NLRs can form high molecular weight complexes called inflammasomes which play a central role in the control of pro-inflammatory immune responses mainly by secretion of IL-1β (Franchi et al., 2009; Ishii et al., 2006).

5.1.4.1 Toll-like receptors

TLRs are a family of evolutionary conserved PRRs expressed by a variety of cell types involved in innate immunity (Akira et al., 2001; Janeway and Medzhitov, 2002). They are type I membrane glycoproteins characterized by a cytoplasmic TIR (Toll/Interleukin-1 receptors (IL-1R)) domain and a leucine-rich repeat ectodomain used for pathogen recognition. They are capable of detecting both exogenous PAMPs such as bacterial lipopeptides, lipopolysaccharide (LPS), flagellin, bacterial or viral DNA and endogenous DAMPs released by dying or damaged cells such as High-Mobility-Group-Box 1 (HMGB1), heat shock proteins and β-defensins (Akira et al., 2006). Activation of TLR signaling pathways results in the recruitment of the intracellular adaptor proteins myeloid differentiation primary response protein 88 (MyD88) or toll-like receptor adaptor molecule 1 (TRIF): MyD88 is universally used by all TLRs, except for TLR3, and activates the transcription factor NF-κB and MAP kinase signaling to induce the expression and release of various pro-inflammatory

cytokines and chemokines. By contrast, TRIF is used by TLR3 and TLR4, which uniquely uses both adaptor molecules, and induces the activation of the transcription factors IRF3 and NF-κB and consequently induces type I interferons as well as pro-inflammatory cytokines (Akira et al., 2006; Gay et al., 2014).

Keratinocytes express the cell surface-associated TLRs 1, 2, 4, 5 and 6 and the endosomal TLRs 3 and 9 (Miller and Modlin, 2007) (Figure 3). In addition, TLR7 expression can be induced by TLR3-mediated recognition of dsRNA in keratinocytes (Kalali et al., 2008). TLR2 is the predominant receptor recognizing staphylococcal lipoproteins and lipopeptides (Nguyen and Gotz, 2016; Takeuchi et al., 2000). Binding of *S. aureus* lipoproteins to TLR2 results in heterodimerization of TLR2 with TLR1 or TLR6 which then translates via MyD88 and NF-κB activation into induction of pro-inflammatory cytokine and AMP expression (Kawasaki and Kawai, 2014). Thereby, the degree of acylation of bacterial lipoproteins influences the consecutive immune response (Nguyen and Gotz, 2016). Upon recognition of tri-acylated lipoproteins, TLR2 forms heterodimers with TLR1, whereas TLR2-TLR6 heterodimers recognize di-acylated lipoproteins (Jin et al., 2007; Kang et al., 2009). While Gram-positive bacteria were thought to mainly contain tri-acylated lipoproteins, both di-and tri-acylated lipoproteins were described in *S. aureus* (Asanuma et al., 2011; Tawaratsumida et al., 2009). Interestingly, di-acylated lipoproteins are mainly found in *S. aureus* during stationary phase or in acidic pH suggesting that acylation is largely dependent on environmental conditions (Kurokawa et al., 2012).

Moreover, TLR2 can cooperate with other co-receptors on the cell surface which expands the spectrum of TLR2 ligands. For example, the scavenger receptor CD36 seems to play a role in *S. aureus* recognition by TLR2: CD36-deficient mice display increased susceptibility to *S. aureus* as well as other TLR2 ligands (Hoebe et al., 2005). Moreover, the lipopeptide LP01 produced by *S. epidermidis* induced enhanced antimicrobial defense against *S. aureus* in keratinocytes via the TLR2/CD36 signaling axis (Li et al., 2013).

Interestingly, unsaturated fatty acids from skin can be incorporated into bacterial lipoproteins, which results in amplification of TLR2-dependent immune stimulation (Nguyen et al., 2016). In addition, it has recently been shown that phenol-soluble modulins (PSMs) produced by staphylococci can release lipoproteins from the

bacterial cell envelope leading to increased TLR2 stimulation of host cells (Hanzelmann et al., 2016).

Ligand binding to TLRs must be tightly regulated to ensure appropriate immune and inflammatory responses. Therefore, host and bacteria have evolved several mechanisms that negatively regulate TLR signaling (Askarian et al., 2014; Kawai and Akira, 2010; Rahman and McFadden, 2011). On the host side, expression levels of TLR2 and its co-receptors regulate the sensitivity to several TLR2 ligands. Additionally, soluble decoy TLRs, splice variants for adaptor proteins and miRNAs can inhibit TLR and NF-κB signaling (Kawai and Akira, 2010). On the pathogen side, *S. aureus*, as well as other pathogens, were shown to express TIR-domain-containing proteins, which were shown to inhibit TLR2-mediated NF-κB signaling (Askarian et al., 2014; Wagner et al., 2018; Zou et al., 2014). Further mechanisms of *S. aureus* to counteract TLR-mediated immune activation are described in section 5.2.3.

Since TLRs are essential for protective immunity against infection, inappropriate or malfunctioning TLR responses can contribute to acute and chronic inflammation. During homeostasis self-derived nucleic acids do not activate TLRs due to endolysosomal localization of TLR7 and TLR9 and due to effective degradation of nucleic acids by serum nucleases (Barton et al., 2006). However, LL-37, which is highly abundant in psoriatic skin, was shown to form stabilizing aggregates with DNA and RNA which can subsequently be endocytosed by DCs and activate TLR7 and TLR9 leading to promotion of skin inflammation (Ganguly et al., 2009; Lande et al., 2007). Additionally, responsiveness of TLR2 to *S. aureus* seems to be lower in AD skin due to a TLR2 polymorphism (Arg753GLN) which renders the intracellular signaling domain inactive. This mutation correlated with increased *S. aureus* skin infections and was associated with a more severe phenotype of AD (Bin and Leung, 2016; Brown, 2017). Moreover, many AD patients express lower amounts of TLR2 in skin cells and produce less pro-inflammatory cytokines after TLR2 stimulation compared to healthy humans (Skabytska et al., 2016). In addition to TLR2, also TLR9, NOD1 and NOD2 polymorphisms were described to correlate with an increased risk for AD (Bin and Leung, 2016; Brown, 2017) indicating that sensing of microbial products which are potentially involved in AD pathology involves not just one TLR ligand but a whole array of ligands that signal via different PRRs.

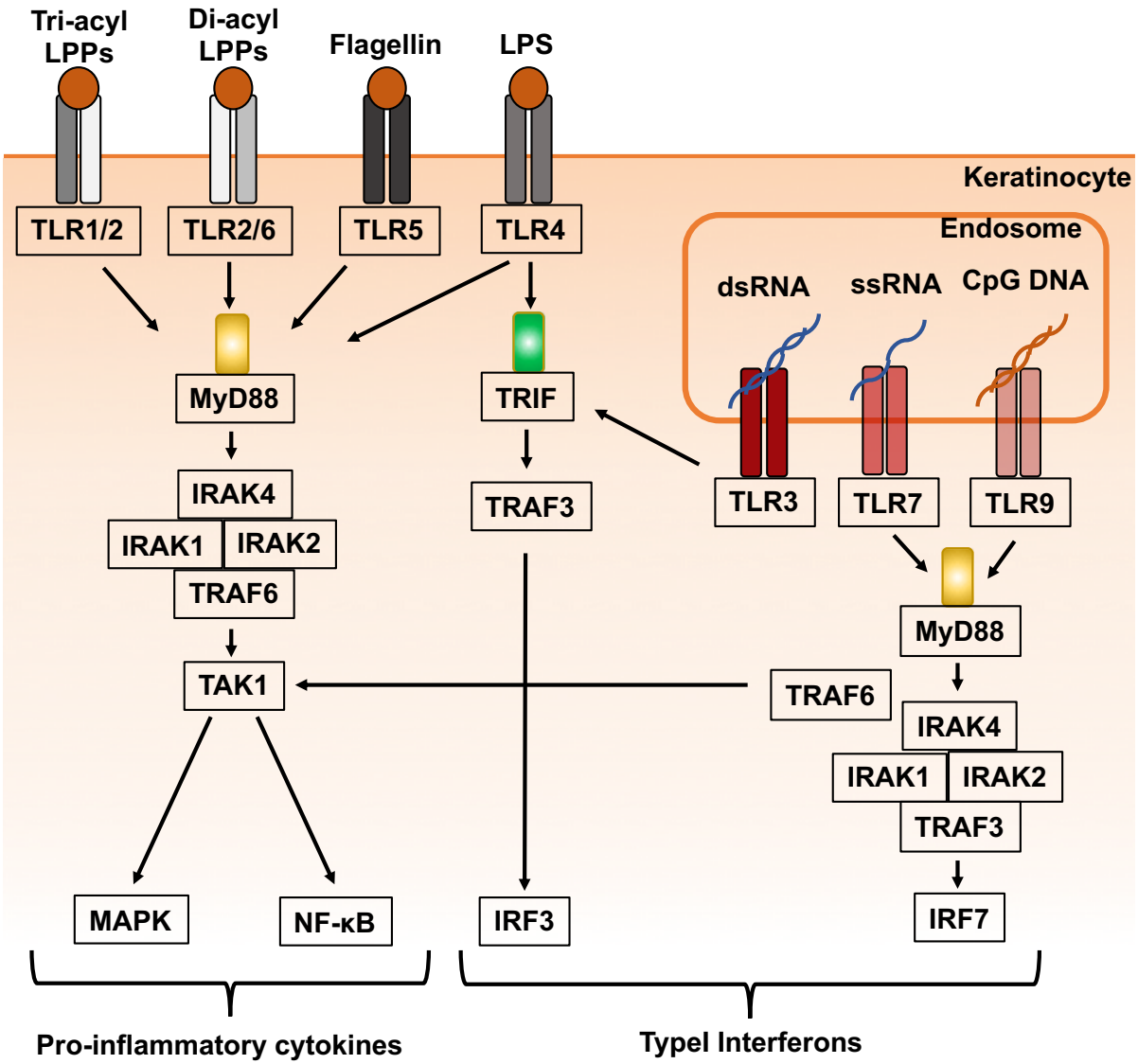


Figure 3: Simplified schematic overview of Toll-like receptors and their signaling networks in human keratinocytes

Depicted are the TLR receptors expressed in human keratinocytes as well as their key adaptors and downstream mediators that are essential for effective host response to infection. Once activated by the indicated ligands, TLRs recruit their specific adapters MyD88 or TRIF. All TLRs universally use MyD88 as an adapter protein, except for TLR3 which uses TRIF. Recruitment of MyD88 results in further recruitment of IRAK4, IRAK2, IRAK1 and TRAF6. Association of IRAK1 and TRAF6 with TAK1 leads to TAK1 activation followed by activation of NF-kB and MAP kinase signaling to induce gene expression of pro-inflammatory cytokines. Endosomal activation of MyD88-dependent TLR signaling, however, results in the activation of IRF7 via TRAF3, leading to transcription of typeI interferons. TRIF-dependent signaling also results in the activation of TRAF3 and concomitant IRF3 activation and transcription of typeI interferons. IRAK = Interleukin-1 receptor-associated kinase; IRF = Interferon regulatory factor; DNA = deoxyribonucleic acid; LPP = Lipoprotein; LPS = Lipopolysaccharide; MyD88 = Myeloid differentiation primary response gene 88; NF-κB = nuclear factor kappa light chain enhancer of activated B cells; dsRNA/ssRNA = double-stranded/ single-stranded ribonucleic acid; TAK1 = Transforming growth factor β activated kinase 1; TLR = Toll-like receptor; TRAF = Tumor necrosis factor receptor associated factor; TRIF = TIR domain containing adapter-inducing interferon-β.

5.1.5 Cytokines and chemokines

Keratinocytes as the initial sensors of environmental stimuli as well as other skin-resident cells are equipped with a variety of cytokines and chemokines which upon release contribute to skin barrier homeostasis and determine subsequent immune reactions. While some cytokines are constitutively expressed in the skin, expression of others can be induced upon injury or infection. TLR stimulation in response to *S. aureus*, for example, results in NF-κB activation and production of pro-inflammatory cytokines and chemokines such as IL-1, IL-6, TNFα or CXCL8 resulting in neutrophil recruitment and AMP production (Figure 4a) (Krishna and Miller, 2012). Deregulated cytokine expression, however, can contribute to epidermal barrier dysfunction as it is observed in AD. In the following the major cytokines and chemokines of the skin are described in more detail. Additionally, the role of cytokines and chemokines during *S. aureus* skin infection as well as during inflammatory skin diseases is depicted in Figure 4.

5.1.5.1 Cytokines

IL-1 family cytokines play a major role in skin homeostasis and immunity. IL-1α is constitutively expressed in keratinocytes and stored in the epidermis (Hauser et al., 1986; Kupper, 1990). Thus the epidermis is shielded by IL-1α which can either be passively released in response to injury or actively in response to infection (Figure 4a) (Murphy et al., 2000). Interestingly, in a 3D organotypic skin model IL-1α expression in keratinocytes was shown to be dependent on stimulation of the aryl hydrocarbon receptor with the commensal *S. epidermidis* (Rademacher et al., 2019). IL-1α is active in its pro-form, however, proteolytic processing dramatically increases its biological activity (Afonina et al., 2015). Interestingly, cleavage of IL-1α can be exerted by pro-inflammatory proteases derived from NK cells or neutrophils such as granzyme B or elastase (Figure 4a) (Afonina et al., 2011). Similarly, IL-36 cytokines, which also belong to the IL-1 cytokine family, can be released by keratinocytes upon tissue injury or microbial stimulation. Cleavage of IL-36 by the neutrophil proteases cathepsin G, elastase and proteinase-3 and not by keratinocyte-derived proteases, highly increased the pro-inflammatory effects of IL-36 cytokines on keratinocytes (Figure 4a) (Henry et al., 2016). Therefore, cleavage of these cytokines bears an intrinsic safeguard mechanism where inflammation is only potentiated in the presence of inflammatory immune cell infiltrates and potential harmful inflammation

can be prevented in their absence. During injury, dysbiosis and skin barrier impairment caused by filaggrin deficiency, however, skin inflammation was shown to be driven through keratinocyte-mediated IL-1α release (Archer et al., 2019).

In contrast to IL-1α, IL-1β requires inflammasome-dependent cleavage by caspase-1 in order to be biologically active (Kostura et al., 1989; Thornberry et al., 1992). Thereby, release of active IL-1β requires two signals: The first signal is the priming signal that can be triggered by different infectious or non-infectious threats that result in NF-κB activation followed by transcription and translation of pro-IL-1β. The second signal is the activating signal by which NLR-mediated sensing of PAMPs or DAMPs results in oligomerization of the multi protein signaling complex termed the inflammasome. Finally, recruitment of caspase-1 forms the active inflammasome resulting in caspase-1 cleavage and activation and concomitant IL-1β-driven inflammation (Lamkanfi and Dixit, 2014). Upon intradermal *S. aureus* infection of the skin, the inflammasome is activated and IL-1β secretion results in the recruitment of neutrophils (Brauweiler et al., 2019; Miller et al., 2007).

Tumor necrosis factor α (TNFα) is a pleiotropic and highly inflammatory cytokine that induces a wide range of other immune cell–attracting cytokines and chemokines as well as AMPs. TNFα can be released in high amounts in response to bacteria-mediated TLR activation and thus plays a huge role in host defense. Binding of TNFα to its receptors activates the two major transcription factors activator protein 1 and NF-κB, that in turn induce genes involved in inflammatory responses (Waters et al., 2013). In response to *S. aureus* skin infection as well as tape-stripping TNFα is upregulated in murine skin (Wanke et al., 2013). Moreover, due to its highly inflammatory capacity, different biologics targeting TNFα are commonly used in the treatment of chronic inflammatory diseases such as psoriasis (Kurschus and Moos, 2017; Sabat et al., 2019).

IL-2 family cytokines include IL-4, IL-13 and IL-21, the prototypic T_H2 cytokines which have been extensively studied in the pathogenesis of AD (Figure 4b). Expression of these cytokines is strongly up-regulated in the skin of AD patients (Neis et al., 2006) as well as in mouse skin upon tape-stripping (Jin et al., 2009). Treatment of keratinocytes with IL-4 in combination with IL-13 leads to a reduced expression of skin barrier proteins such as filaggrin, loricrin and involucrin (Cornelissen et al., 2012; Howell et al., 2009). Mice treated with recombinant IL-4

show a reduced epidermal thickness (Hatano et al., 2013) whereas mice overexpressing IL-4 or IL-13 in keratinocytes, spontaneously develop an inflammatory skin reaction that displays many features of AD including *S. aureus* colonization (Chan et al., 2001; Zheng et al., 2009). In line with this, IL-4-ko mice develop a strengthened skin barrier and are protected from the development of STAT6-induced allergic skin inflammation (Sehra et al., 2010).

IL-1 cytokines and TNFα cytokines as well as TLR activation are also major activators of **IL-6 cytokine** expression (Hunter and Jones, 2015). Concurrently, IL-6 deficiency leads to impaired immunity against microbial infection (Kopf et al., 1994; Neveu et al., 2009; van der Poll et al., 1997) and children with inhibitory autoantibodies against IL-6 develop recurrent staphylococcal infections (Puel et al., 2008). Similarly, patients with STAT3 mutations, which are common among AD patients (Weidinger et al., 2018), show impaired IL-6 activity and are susceptible to recurrent staphylococcal skin infections (Freeman and Holland, 2010). Additionally, IL-6 is involved in wound healing (Lin et al., 2003) and keratinocyte proliferation (Sato et al., 1999). Another member of the IL-6 cytokine family is IL-31 which induces AMP expression in an IL-1α-dependent manner during skin homeostasis (Hanel et al., 2016). Since IL-31 is mainly produced by T_H2 cells but its receptor is expressed by a wide range of cells including keratinocytes this implies a role for IL-31 in AD pathogenesis (Kato et al., 2014). Indeed, AD patients show significant higher levels of IL-31 mRNA in serum and lesional skin samples (Kim et al., 2011). Moreover, IL-31 interferes with keratinocyte differentiation and represses filaggrin expression (Cornelissen et al., 2012). Clinical trials have revealed that administration of an anti-IL-31 receptor antibody significantly alleviates pruritus in patients with AD (Furue et al., 2018).

IL-17 family cytokines comprise six members, IL-17A-F (Gaffen, 2011). IL-17A and IL-17F are the signature cytokines for T_H17 cells (Yao et al., 1995) but the main producers also include $CD8^+$ cytotoxic T cells as well as $T_{\gamma\delta}$ cells and ILC3s (Kurschus and Moos, 2017). Keratinocytes, on the other hand, mainly produce IL-17C in response to pro-inflammatory and bacterial stimuli including *S. aureus* (Ramirez-Carrozzi et al., 2011; Roth et al., 2014). The receptor for IL-17A/F, however, is expressed quite ubiquitously including in keratinocytes, whereas IL-17RE, the receptor for IL-17C, is rather expressed on non-hematopoietic cells such

as keratinocytes (Kurschus and Moos, 2017). Thereby, the responses of keratinocytes to IL-17C and IL-17A/F are very similar (Ramirez-Carrozzi et al., 2011). IL-17 ligand binding to the IL-17 receptor results in NF-κB and ERK activation which ultimately leads to the expression of other pro-inflammatory cytokines and chemokines such as IL-6, IL-36 and CXCL8 as well as AMP production. Therefore, IL-17 cytokines induce a host response and initiate neutrophil infiltration into infected tissues. Thereby, IL-17 signaling is important in cutaneous defense against several bacteria and fungi (Li et al., 2018) and major roles have been described for *S. aureus* (Cho et al., 2010). Despite its beneficial effects in host defense, IL-17 is also implicated in several inflammatory diseases such as psoriasis where the IL-17 signaling axis is crucial for pathogenesis (Figure 4c) (Furue and Kadono, 2019) and targeting monoclonal antibodies are widely approved for therapy (Kurschus and Moos, 2017). Since IL-17 was shown to act in synergy with other pro-inflammatory cytokines such as TNFα (Ruddy et al., 2004) or IL-1β (Iyoda et al., 2010) the main function of TNFα-targeting antibodies in psoriasis may be the inhibition of synergy with IL-17. Additionally, synergistic AMP induction of IL-22 and IL-17 are the main cause for impaired keratinocyte cornification observed in psoriasis (Wolk et al., 2006). Finally, IL-17C neutralization has been shown to ameliorate skin inflammation in mouse models both of psoriasis and AD (Vandeghinste et al., 2018).

Other cytokines important in skin immunity include interferons which are mainly involved in anti-viral immune defense, members of the IL-12 cytokine family such as IL-23 as well as regulatory cytokines such as IL-10 (Hanel et al., 2016).

5.1.5.2 Chemokines

Chemokines are small chemotactic molecules that can be classified into four groups based on the position of the conserved cysteine residues: CXC, CC, C and CX_3C (Zweemer et al., 2014). Chemokines typically interact with G protein-coupled chemokine receptors by which diverse signals leading to leukocyte migration, inflammation and differentiation are transmitted (Nourshargh and Alon, 2014; Tan et al., 2015).

CXCL1, CXCL2 and CXCL8 primarily recruit neutrophils to the site of inflammation by binding to the chemokine receptors CXCR1 and CXCR2 (Rajarathnam et al., 2019). CXCL8 expression is mainly regulated by NF-κB activation in response to TLR2 activation but expression can also be induced by pro-

inflammatory cytokines such as TNFα and IL-1 cytokines (Krishna and Miller, 2012). Rodents lack CXCL8 but the chemokines keratinocyte chemoattractant (KC), macrophage inflammatory protein 2 (MIP-2) and LPS-induced CXC-chemokine (LIX) are regarded as functional homologues since they represent closely related chemokines that effectively recruit neutrophils (Zlotnik and Yoshie, 2000).

Moreover, keratinocytes are important sources of T cell recruiting chemokines in the skin: By expressing CXCL9, CXCL10, and CXCL11 activated keratinocytes attract different subtypes of T cells into the skin and thereby orchestrate skin inflammation (Nestle et al., 2009). Furthermore, commensal-induced CCL20 expression in keratinocytes at the hair follicles was shown to drive T_{reg} migration into neonatal mouse skin which leads to the establishment of tolerance towards commensals (Scharschmidt et al., 2017; Scharschmidt et al., 2015).

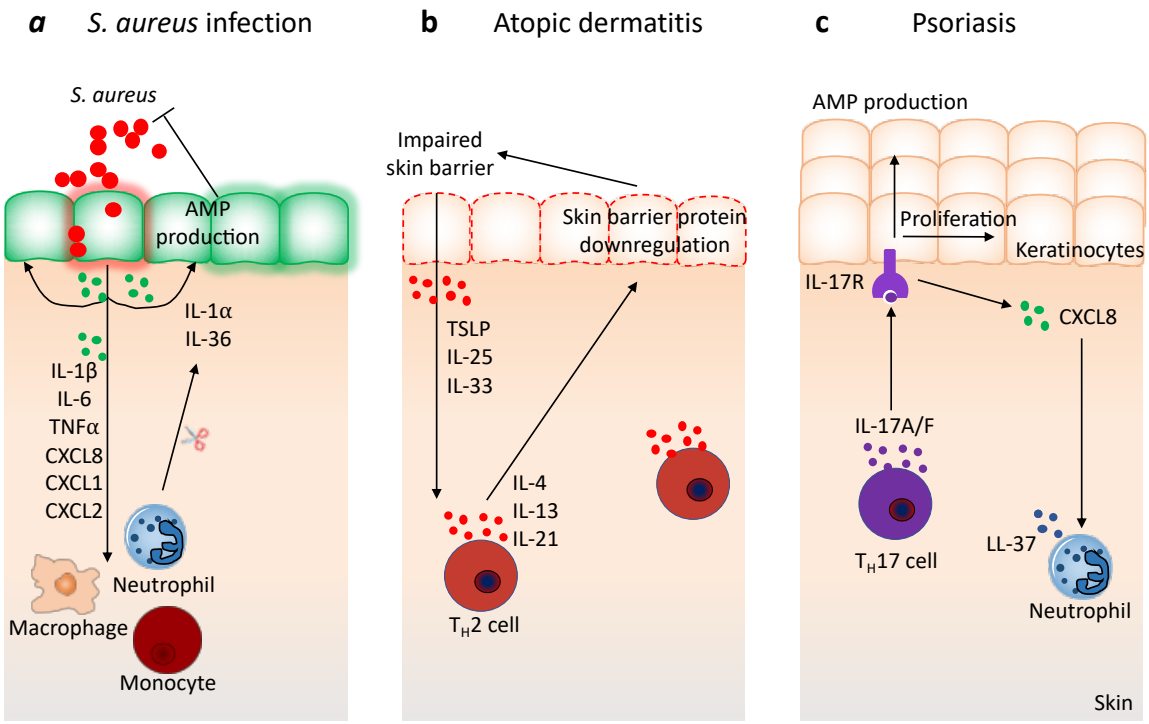


Figure 4: The role of cytokines and chemokines during *S. aureus* infection and inflammatory skin diseases

a) The epidermis is shielded by keratinocyte-mediated IL-1α production (green shield) which can either be passively released in response to injury or actively in response to infection. Upon *S. aureus* infection of the skin, diverse pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, TNFα, CXCL8, CXCL2 and CXCL1 are secreted by keratinocytes in order to recruit phagocytic cells to clear the infection. At the same time neutrophil-derived proteases can cleave IL-1α and IL-36 into their active forms to potentiate keratinocyte-mediated paracrine pro-inflammatory signaling. Pro-inflammatory cytokines can in turn induce the expression of AMPs in keratinocytes. b) In response to an impaired skin barrier (dashed red line), keratinocyte-mediated secretion of cytokines such as TSLP, IL-25 and IL-33 drive T_H2 inflammation. T_H2 cytokines lead to the downregulation of skin barrier proteins such as filaggrin, loricrin and involucrin by keratinocytes thus starting a vicious cycle. c) During psoriasis skin-infiltrating T_H17 cells secrete T_H17 cytokines such as IL-17A/F. Keratinocytes do not express IL-17A/F cytokines but their receptor components. Binding of IL-17A/F to the IL-17R results in the expression of AMPs and chemokines such as CXCL8 leading to neutrophil recruitment to the skin where they secrete LL-37 and initiate a pro-inflammatory loop. In addition, IL-17 cytokines induce aberrant differentiation of keratinocytes resulting in hyperproliferation and thickening of the epidermis. IL = Interleukin; CXCL = C-X-C motif ligand; TNFα = Tumor necrosis factor α; TSLP = thymic stromal lymphopoietin; AMP = Antimicrobial peptide.

5.2 The skin microbiome

Our body contains an estimated composition of 10^{12} microbes (Ley et al., 2006) in the gut and 10^{10} in the skin (Grice et al., 2008). However, compared to the gut, our skin provides poor nutrient availability to its colonizing microbes. Therefore, the resident microbiota has adapted and utilizes the limited resources present in our sweat, sebum and stratum corneum. Colonizing staphylococci for example have evolved diverse strategies to ensure survival on the skin. In addition to being halo-tolerant, they can use the urea in the sweat as a nitrogen source. Moreover, staphylococci express proteases that liberate nutrients from the stratum corneum. Interestingly, colonizing malassezia and corynebacteria are unable to produce their own lipids and utilize the lipid compounds present in the sebum and stratum corneum. Therefore, compared to other fungi, malassezia genomes are enriched in genes encoding for lipid metabolism and genes involved in carbohydrate metabolism are depleted. Moreover, by releasing proteases and lipases propionibacteria manage to release arginine from skin proteins and lipids from the sebum (Scharschmidt and Fischbach, 2013).

5.2.1 Skin microbiome composition

The majority of skin bacteria belong to the three phyla of Actinobacteria, Firmicutes and Proteobacteria with the most common colonizers corynebacteria, propionibacteria and staphylococci (Costello et al., 2009; Human Microbiome Project, 2012; Oh et al., 2016). However, different skin sites provide different microenvironments that largely vary in UV exposure, pH, temperature, moisture, sebum content and topography (Grice and Segre, 2011). Presence of different appendages such as sebaceous glands or sweat glands on different body sites largely shape the composition of the local microbiota. Consequently, the different skin sites can be broadly grouped into three categories: oily or sebaceous skin sites such as the face, chest and back, moist skin sites such as the bend of the knee or the elbow and dry skin sites such as the forearm and palms. Additionally, sweat and sebum also contain antimicrobial molecules such as free fatty acids and AMPs that also shape the microbial composition.

The first group to thoroughly analyze the composition of the skin microbiota used 16S ribosomal RNA gene sequencing to identify the specific microbes present on the

skin (Costello et al., 2009). The results showed that in healthy adults, the composition of the skin microbiota was primarily dependent on the physiology of the individual skin sites (Figure 5). Interestingly, the composition of the microbiota was shown to vary less among different people than among different body sites of one individual. Furthermore, sebaceous sites displayed a low diversity in microbe composition and were dominated by propionibacteria, whereas moist skin sites were diversely colonized by staphylococci and corynebacteria. The most diversely colonized sites, however, were the dry skin sites which also exhibited a high abundance of Gram-negative species and interestingly even exhibited a higher bacterial diversity than the gut (Costello et al., 2009). Later, the findings of Costello et al. (2009) that microbiota is mainly specific to skin site were confirmed by other groups using more advanced sequencing methods such as whole metagenomics sequencing (Findley et al., 2013; Grice et al., 2009; Oh et al., 2014; Oh et al., 2016). Additionally, metagenomic analysis revealed that bacteria make up around 75% of our skin microbiota while fungi are the least abundant with only 1%. In contrast to bacteria, fungi composition does not differ across different body sites (Findley et al., 2013; Oh et al., 2016). Moreover, around 15% of our skin microbiome consists of viruses which were shown to be specific to the individual and not to the body site (Oh et al., 2016). However, due to the lack of reference genomes among viruses and the disregard of RNA viruses by metagenomics a common virome among individuals has not been identified yet (Byrd et al., 2018; Hannigan et al., 2015; Oh et al., 2016).

Initial skin colonization in newborns was shown to be undifferentiated across different body sites and depend on the delivery mode (Dominguez-Bello et al., 2010). However, site specificity was shown to be established already within the first two days after birth (Kennedy et al., 2017; Meylan et al., 2017). During puberty, the composition of the skin microbiota undergoes reconstruction. Increasing levels of hormones stimulate sebaceous glands to produce higher amounts of sebum which in turn favors the colonization of lipophilic bacteria such as propionibacteria. In line with this observation, the skin microbiota of infants is characterized by a high abundance of staphylococci (Oh et al., 2012). Therefore, AD, which is often associated with *S. aureus* colonization, is more prevalent in infants and declines with increasing age (Hanifin et al., 2007; Harrop et al., 2007).

Furthermore, longitudinal sampling of skin microbiota of healthy adults demonstrated a high stability of microbial communities over a 2-year period, despite

constant environmental changes (Oh et al., 2016). Analysis at the strain level showed that this stability was determined by the maintenance of strains over time rather than by acquisition of new strains from the environment (Oh et al., 2016). Similarly, longitudinal studies of the gut microbiome also showed that individual strains persist over time (Faith et al., 2013; Schloissnig et al., 2013). Describing microbial communities at the strain level becomes especially important when conclusions on functional effects on the host are drawn. Virulence genes, for example, which are associated with biofilm formation or antibiotic resistance, were shown to be variably present in some *S. epidermidis* strains (Conlan et al., 2012; Meric et al., 2018).

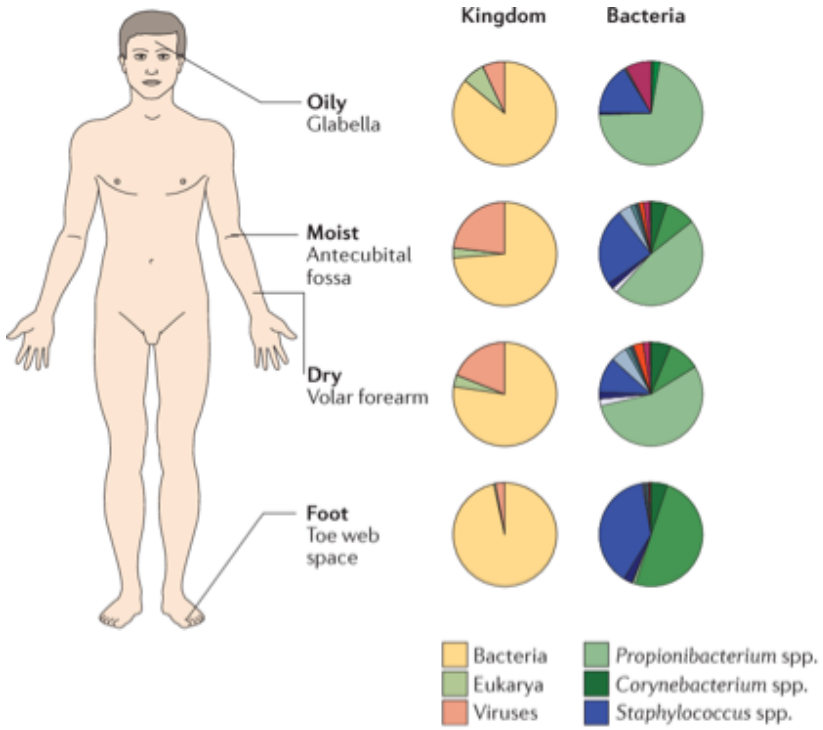


Figure 5: The skin microbiome composition is primarily dependent on the physiology of distinct skin sites. Adapted from (Byrd et al., 2018)

Four sites are shown to represent the major microenvironments of the skin: forehead/glabella (sebaceous); antecubital fossa (moist); volar forearm (dry); and toe web space (foot). Pie charts represent the relative abundance of bacteria, eukarya and viruses among healthy adults. Additionally, major bacterial species are shown in relative abundance plots. Unlabeled colors represent other bacterial species

5.2.2 Coagulase-negative Staphylococci

Since microbial communities have been explored for a long time by using culture-based techniques, microorganisms that thrive under common culture conditions such as staphylococci have been isolated more frequently than anaerobic growing bacteria. In humans, CoNS, especially *S. epidermidis* but also *S. capitis*, *S. warneri*,

S. hominis and *S. lugdunensis*, are the most frequently isolated skin commensals (Becker et al., 2014). *S. epidermidis* particularly colonizes moist areas such as the axillae, inguinal and perineal areas, anterior nares, conjunctiva and toe webs (Kloos and Musselwhite, 1975; Schleifer and Kloos, 1975). *S. epidermidis* does not produce aggressive toxins and many positive effects on skin homeostasis and immunity are attributed to this commensal (Stacy and Belkaid, 2019).

Also *S. lugdunensis* was shown to be part of the normal skin flora where it particularly colonizes the inguinal and perineal regions, in the groin area, the axillae as well as the nail bed and the nose (Bieber and Kahlmeter, 2010; Zipperer et al., 2016). *S. lugdunensis* can co-exist with other commensals on human skin such as *S. epidermidis* (Bieber and Kahlmeter, 2010; Gong et al., 2006), but intriguingly, nasal colonization by *S. aureus* or *S. lugdunensis* (Bieber and Kahlmeter, 2010; Zipperer et al., 2016) was shown to be mutually exclusive due to production of lugdunin, a peptide antibiotic (Zipperer et al., 2016). In fact, humans who are colonized by *S. lugdunensis* in the nose have a 6-fold lower risk of *S. aureus* carriage than individuals who are not colonized (Zipperer et al., 2016).

However, due to their ubiquitous presence on human skin, commensals can also act as opportunistic pathogens: *S. epidermidis,* for example, frequently causes nosocomial infections mainly by colonizing medical devices where it causes catheter infections (Otto, 2009b). Additionally, *S. epidermidis* is one of the most frequent causes of sepsis in newborns (Dong et al., 2018). Nosocomial *S. epidermidis* infections are associated with methicillin resistance, inflammatory phenol-soluble modulin PSM-mec and polysaccharide production (Otto, 2009b). Recently, strain-specific differences among commensal and invasive *S. epidermidis* strains were identified (Conlan et al., 2012; Meric et al., 2018): Using pangenome-wide association studies, sequence variations in 61 *S. epidermidis* genes associated with biofilm formation, cell toxicity, methicillin resistance and CXCL8 production were identified. In conclusion, enrichment of these putative virulence determinants among infectious isolates suggests that pathogenic strains are a subset of the commensal population. Additionally, *S. aureus* and *S. epidermidis* were shown to share genes involved in pathogenicity suggesting horizontal gene transfer (Meric et al., 2018). Similarly, *S. lugdunensis* is frequently associated with nosocomial infections (Argemi et al., 2019). In addition to nosocomial infections of medical devices, *S. epidermidis* was shown to increasingly colonize the skin of AD patients during flares (Byrd et al.,

2017; Kong et al., 2012). However, whether *S. lugdunensis* colonization is also increased in AD patients remains to be evaluated.

5.2.3 *Staphylococcus aureus*

S. aureus is a Gram-positive bacterium that frequently colonizes the anterior nares of 30% of the human population (Wertheim et al., 2005). However, whether *S. aureus* should be considered a commensal is controversial, because at the same time, *S. aureus* is also a leading cause of endocarditis, bacteremia, osteomyelitis and skin and soft tissue infections (DeLeo et al., 2010). Since 80% of the *S. aureus* strains isolated from the blood of bacteremia patients match the strain present in their noses, the risk of developing *S. aureus* infections is higher for individuals that carry *S. aureus* in their nose (von Eiff et al., 2001; Weidenmaier et al., 2012). Eradication of nasal *S. aureus* significantly prevents the susceptibility for subsequent infections (Bode et al., 2010). Additionally, *S. aureus* is frequently isolated from inflamed and non-inflamed skin of AD patients where it further contributes to skin inflammation (see section 5.2.3.2) (Kong et al., 2012).

Opposed to its CoNS counterparts, *S. aureus* was shown to express two coagulases, staphylocoagulase and von Willenbrand factor, which convert fibrinogen to fibrin resulting in blood clotting upon infection (Bjerketorp et al., 2002; Kinoshita et al., 2008). This reaction has been used for a long time to differentiate coagulase-positive from negative staphylococci (Needham et al., 1945). Even though CoNS and *S. aureus* belong to the same genus their effects on the host are antagonistic. The success of *S. aureus* in being such a potent pathogen largely resides in the myriad of different surface-associated as well as secreted virulence factors it produces. Additionally, increasing usage of antibiotics gave rise to methicillin-resistant *S. aureus* strains (MRSA) which, by horizontal gene transfer, have acquired the mecA gene that confers resistance to the entire class of beta-lactam antibiotics (Hartman and Tomasz, 1984). Since the first clinical observation of MRSA in the 1960s different healthcare- and community-associated (HA-MRSA and CA-MRSA) MRSA stains have been observed across the globe (Turner et al., 2019). Due to asymptomatic nasal carriage, MRSA carriage of is often unrecognized which can become hazardous when these patients undergo surgery, immunosuppression or suffer from trauma which usually puts patients at a higher risk of developing invasive infections caused by endogenous pathogens (Wertheim et al., 2005). Among CA-

MRSA strains the USA300 strain has risen to become the predominant strain for skin and invasive infections in North America (Diekema et al., 2014). In addition to methicillin resistance this strain is characterized by an increased presence of virulence genes (Planet, 2017).

5.2.3.1 *S. aureus* virulence factors

S. aureus produces a large array of virulence factors that contribute to its pathogenesis including many proteins that mediate adhesion to epithelial surfaces, destruction of host cells and tissues and virulence factors by which *S. aureus* circumvents elimination by the innate and adaptive immune system of the host (Guerra et al., 2017; Lowy, 1998) (Figure 6). In fact, the frequent recurrence of *S. aureus* infections demonstrates its ability to manipulate host immune responses. In the following the major virulence factors involved in *S. aureus* skin infection are described:

S. aureus is equipped with a large array of surface proteins that mediate adhesion to epithelial surfaces: These proteins, referred to as **microbial surface components recognizing adhesive matrix molecules (MSCRAMMs)**, specifically bind to skin epithelial surface proteins such as cytokeratin and loricrin (clumping factor B ClfB). Iron-regulated surface determinant A (IsdA) also promotes adhesion to loricrin, involucrin and cytokeratin. In addition, fibronectin-binding proteins A and B (FnBP) bind to the extracellular matrix proteins fibronectin (Foster and Hook, 1998; Patti et al., 1994). Interaction of fibronectin and integrin a5b1 can result in *S. aureus* invasion into epithelial cells (Foster et al., 2014). Moreover, *S. aureus* wall teichoic acid (WTA) binding to a type-F scavenger receptor, termed SR-F1 (SREC-I) in the nasal cavity plays an important role in nasal epithelial colonization (Wanner et al., 2017).

Moreover, *S. aureus* secretes multiple different **proteases** that destroy the epithelial barrier and thus promote its colonization: The exfoliative toxin is a protease that degrades desmoglein, thereby disrupting the cell-cell contacts between keratinocytes (Amagai et al., 2000). *S. aureus* V8 protease induces severe transepidermal water loss in an epicutaneous mouse model by disturbing the stratum corneum (Hirasawa et al., 2010). *S. aureus* was shown to produce at least ten proteases which enables epidermal penetration. In addition, T_H2 cytokines as well as absence of filaggrin, which are both hallmarks of AD, facilitated protease activity (Nakatsuji et al., 2016). Moreover, stimulation of endogenous keratinocyte proteases

further contributes to barrier destruction (Williams et al., 2017). In addition to epithelial barrier destruction, several of the *S. aureus* proteases can cleave and inactivate AMPs such as dermcidin or LL-37 (Lai et al., 2007; Sieprawska-Lupa et al., 2004). In addition to proteases, secretion of other enzymes such as lipases can substantially contribute to *S. aureus* virulence (Nguyen et al., 2017).

S. aureus also secretes many **toxins** that directly damage host cells (Otto, 2014): Panton-Valentine leukocidin, for example, damages leukocytes and keratinocytes (Chi et al., 2014). Moreover, *S. aureus* **phenol-soluble modulins (PSMs)** were shown to exhibit cytolytic activities at high concentrations (Peschel and Otto, 2013). PSMs exhibit pro-inflammatory properties at nanomolar concentrations (Peschel and Otto, 2013; Wang et al., 2007) by stimulating the formyl-peptide receptor 2 (FPR2), which is expressed by different types of immune cells and keratinocytes (Kretschmer et al., 2010). PSMα was shown to induce pro-inflammatory cytokines from keratinocytes and triggers skin inflammation in response to epicutaneous *S. aureus* infection (Nakagawa et al., 2017). PSMs are also used by *S. aureus* to lyse neutrophils and escape from killing upon phagocytosis (Geiger et al., 2012; Surewaard et al., 2013). One of the various *S. aureus* PSMs, the δ-toxin, also contributes to skin inflammation by activating mast cells (Nakamura et al., 2013). Moreover, PSMs mobilize the release of lipoproteins from the *S. aureus* cytoplasmic membrane thereby promoting TLR2 activation (Hanzelmann et al., 2016).

Additionally, several staphylococcal **superantigens** such as toxic shock syndrome toxin 1 (TSST-1) or staphylococcal enterotoxins (SE) also contribute to *S. aureus* pathogenesis during invasive infections as well as in AD (Skov et al., 2000; Spaulding et al., 2013).

S. aureus is able to **circumvent** host **innate defense** by various **escape mechanisms** such as PRR signaling inhibition or resistance to host AMPs: The latter can be achieved, for example, by modification of cell wall molecules with positively-charged amino acids and concomitant reduction of their affinity for AMPs (Peschel and Sahl, 2006). In order to escape TLR2 recognition, *S. aureus* secretes the TLR2 antagonist staphylococcal superantigen-like protein 3 (SSL3) which prevents bacterial lipoproteins from binding to TLR2 (Koymans et al., 2015; Yokoyama et al., 2012). Moreover, the *S. aureus* TIR domain protein blocks TLR2-mediated NF-κB signaling by sequestering MyD88 (Askarian et al., 2014). Additionally, skin exposure

to TLR2-6 lipopeptides from *S. aureus* was shown to suppress immune responses via induction of regulatory myeloid cells (Skabytska et al., 2014). Many *S. aureus* strains produce chemotaxis inhibitory proteins (**CHIPS)** and formyl peptide receptor-like 1 inhibitory (**FLIPr**) and **FLIPr-like** protein all of which block formyl peptide receptors und thus inhibit leukocyte migration towards the site of infection (Bloes et al., 2015; de Haas et al., 2004).

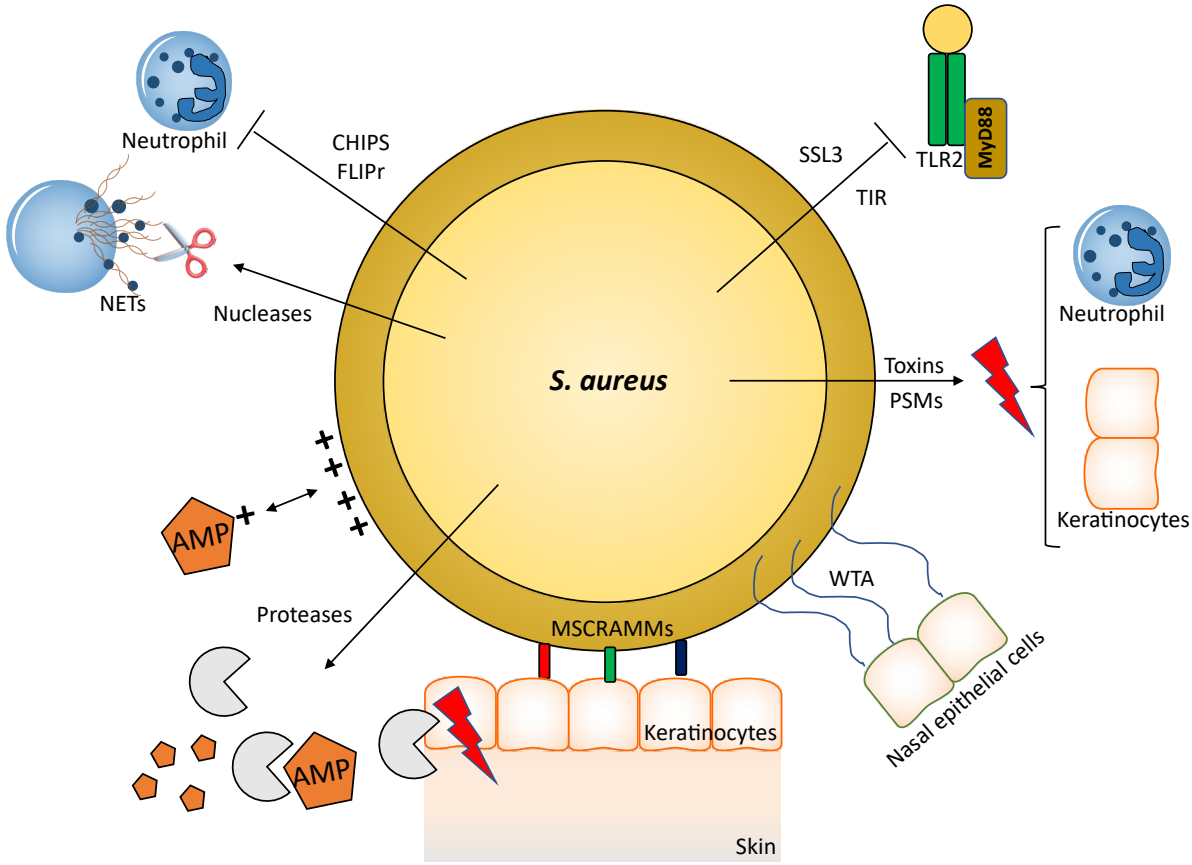


Figure 6: *S. aureus* virulence factors influencing epithelial tissue infection

S. aureus produces a large array of virulence factors that contribute to its pathogenesis. Eclectic virulence factors such as proteins that mediate adhesion to epithelial surfaces, destruction of host cells and tissues as well as virulence factors by which *S. aureus* circumvents elimination by the innate and adaptive immune system of the host are described in this figure. CHIPS = chemotaxis inhibitory proteins; FLIPr = formyl peptide receptor-like 1 inhibitory; NET = neutrophil extracellular traps; AMP = antimicrobial peptide; MSCRAMM = microbial surface components recognizing adhesive matrix molecules; WTA = wall teichoic acid; PSM = phenol-soluble modulin; TIR = Toll/Interleukin-1 receptor; SSL3 = staphylococcal superantigen-like protein 3; TLR2 = Toll-like receptor 2; MyD88 = Myeloid differentiation primary response 88.

5.2.3.2 *S. aureus* and atopic dermatitis

S. aureus skin colonization is highly associated with AD, with prevalence ranging from 30-100% depending on the age of the patient, sample size or sampling method (Tauber et al., 2016; Totte et al., 2016; Travers et al., 2010). Even though *S. aureus* has many surface proteins mediating adhesion to cytokeratin, loricrin, or involucrin of corneocytes in the stratum corneum (Weidenmaier et al., 2012), healthy skin does not appear to offer favorable conditions for persistent skin colonization whereas barrier disruption of murine skin increases *S. aureus* colonization (Wanke et al., 2013). The reasons have remained elusive but it is likely that alterations in available nutrients and in skin microbiome composition associated with barrier defects are necessary for *S. aureus* to thrive on human skin (Kong et al., 2012). In a longitudinal study of pediatric individuals with AD it was shown that during the flares of AD *S. aureus* levels on the skin increase up to 100-fold compared to normal skin. 16S rRNA and whole genome sequencing showed that especially the relative abundance of staphylococci, mainly *S. aureus* but intriguingly also *S. epidermidis,* increased during the flare versus the pre- or post-flare state. Moreover, the increase in relative abundance of staphylococci correlated with a more severe disease (Byrd et al., 2017; Kong et al., 2012). On the strain level, it was shown that AD patients were colonized with heterogenous *S. epidermidis* strains, but *S. aureus* strains were clonal (Byrd et al., 2017).

However, the key question to which answers are still conflicting remains: Does *S. aureus* cause AD or is *S. aureus* colonization a result of AD? Kennedy et al. (2017) describe a study of 50 infants who were swabbed at 4 different skin sites at 6 different time points starting from birth to the age of 2 for a 2-year period. Using 16S rRNA gene sequencing they could not detect any significant increase in *S. aureus* colonization in the 10 infants that developed AD. Therefore, they concluded that the onset of AD precedes *S. aureus* colonization (Kennedy et al., 2017). By contrast, in a larger longitudinal study by Meylan et al. (2017) 149 infants, 36 of which developed AD, were sampled 7 times from birth to the age of 2 *S. aureus* was detected before the onset of AD. Prevalence of *S. aureus* at 3 months of age was significantly higher in infants who subsequently developed AD. Interestingly, prevalence of the CoNS *S. hominis* at 3 months of age was negatively correlated with subsequent AD development. Therefore, they conclude that CoNS contribute to skin homeostasis and protection against infection (Meylan et al., 2017). Further experimental evidence

that *S. aureus* can promote AD in susceptible individuals can be found in the literature: *S. aureus* was shown to stimulate keratinocytes to increase endogenous kallikrein protease activity which can damage the epithelial barrier by degradation of desmoglein-1 and filaggrin (Williams et al., 2017). Furthermore, epidermal penetration of *S. aureus* and concomitant induction of T_H2 cytokines in murine skin and human skin equivalents was shown to correlate with *S. aureus* protease activity (Nakatsuji et al., 2016). Moreover, *S. aureus* lipopeptides where shown to induce the production of thymic stromal lymphopoietin (TSLP), which is considered an initiator of AD, in keratinocytes (Vu et al., 2010). In an epicutaneous mouse skin colonization model staphylococcal δ-toxin was identified as responsible for the enhanced IgE and IL-4 production and concomitant inflammation enhancement. Additionally, it was shown that *S. aureus* strains isolated from AD patients produce large amounts of δ-toxin (Nakamura et al., 2013). Comparison of laboratory strains of *S. aureus* and clinical isolates from AD skin revealed that AD strains alter the T cell responses via Langerhans cells, resulting in T_H2-shifted immune responses suggesting that the colonized AD strain on the skin surface itself may promote an AD-specific immune environment (Iwamoto et al., 2017). In line with this Byrd et al. (2017) showed biological effects of *S. aureus* at the strain level: topical colonization of mice with strains isolated from AD patients induced higher levels of skin inflammation and immune signatures characteristic for AD patients such as T_H2 cell expansion (Byrd et al., 2017). By sequencing different *S. aureus* strains can be grouped into different clonal complex (CC) groups. Recent evidence shows that AD skin mainly harbors strains of the CC1 type while CC30 was associated with healthy individuals (Harkins et al., 2018b; Iwamoto et al., 2019; Rojo et al., 2014). Additionally, temporal variation in *S. aureus* CC type was linked to AD flares (Clausen et al., 2019). Further analyses of specific *S. aureus* strains of AD patients over time are needed to classify the characteristics of AD strains, leading to the discovery of the distinct markers of AD-derived *S. aureus*. Additionally, reduced presence of AMPs such as LL-37, dermcidin and β-defensins also contribute to *S. aureus* colonization in AD patients (Rieg et al., 2005; Yamasaki and Gallo, 2008). Ultimately, the T_H2 cytokines IL-4 and IL-13 were shown to inhibit hBD2 and hBD3 gene expression (Hata et al., 2010). Taken together, these recent data support the hypothesis that *S. aureus* can promote AD development in susceptible individuals, however, the detailed mechanisms still remain elusive.

5.3 Functional effects of skin microbiota

Members of the microbiota can directly and dynamically interact with pathogens and immune cells and the results of this interaction can define the pathogenesis and the outcome of a given infection. Before detection by PRRs and phagocytosis by leukocytes, pathogens are faced with a complex community of commensal microorganisms that reside on the skin and prevent pathogen colonization by multiple mechanisms: By inhabiting their various niches, commensals prevent pathogenic bacteria from gaining access to nutrients. This process is also referred to as colonization resistance and provides a first level of protection against invading pathogens (Buffie and Pamer, 2013; Sassone-Corsi and Raffatellu, 2015). On a second level, commensal bacteria can directly target pathogenic bacteria by producing bacteriocins or other peptide antibiotics that inhibit or kill competing bacteria. Ultimately, by triggering innate and adaptive immune responses in the host, commensal bacteria provide protection against invading pathogens. In the following the direct functional effects of CoNS on *S. aureus* skin infection as well as the effects of CoNS on skin immunity are described.

5.3.1 Direct effects of skin microbiota on pathogens

CoNS are frequent producers of lanthionine-containing **bacteriocins**, so called **lantibiotics**. These peptides contain the unusual amino acids lanthionine or 3-methyl-lanthionine (Dischinger et al., 2014; Schnell et al., 1988; Willey and van der Donk, 2007). Lantibiotics such as nisin and (pro)-gallidermin are produced by the commensals *Lactococcus lactis* and *S. gallinarum* (Kellner et al., 1988). Additionally, it was recently shown that the skin commensal *S. capitis* also contains the gene cluster for gallidermin biosynthesis (Kumar et al., 2017). Interestingly, the amino acid sequence of gallidermin differs only in one amino acid from the *S. epidermidis*-derived lantibiotic epidermin (Kellner et al., 1988). Both nisin and gallidermin share the same binding motif for the cell wall precursor lipid II and consequently both lantibiotics inhibit cell wall biosynthesis of a wide spectrum of Gram-positive bacteria including *S. aureus* (Breukink et al., 2003). Additionally, interaction of the cationic nisin with lipid II also induces pore formation and consequently disruption of ion gradients and the membrane of the anionic bacterial cell wall initiated by the high electrostatic forces (Brotz et al., 1998; Wiedemann et al., 2001). This pore consists of four lipid II molecules and eight nisin molecules (Hasper et al., 2004). The 22 amino

acid short gallidermin, however, is not able to span the membrane and hence its mode of action is primarily cell wall biosynthesis inhibition rather than pore formation. Apart from lipid II, gallidermin was additionally shown to interact with lipid I, III and IV resulting in not only murein but also wall teichoic acid synthesis inhibition (Muller et al., 2012). Due to the protective outer membrane Gram-negative bacteria are usually not affected by lantibiotics (Dischinger et al., 2014). In recent work of the group of Richard Gallo *S. epidermidis* and *S. hominis* strains were shown to produce novel lantibiotics that inhibit the growth of *S. aureus*. Interestingly, these lantibiotic-producing strains were missing in AD patients. However, a single application of lantibiotic-producing *S. epidermidis* and *S. hominis* onto the skin of AD patients reduced the number of colonizing *S. aureus* within 24 hours demonstrating the translational potential of this probiotic strategy (Nakatsuji et al., 2017).

Additionally, *S. epidermidis* was shown to inhibit *S. aureus* nasal colonization and biofilm formation via expression of the **serine protease** Esp (Iwase et al., 2010). It was shown that Esp degrades proteins of *S. aureus* essential for host cell adhesion (Sugimoto et al., 2013). Interestingly, pan genome sequencing of skin commensals revealed that most *S. epidermidis* strains contain the Esp gene *gseA* (Conlan et al., 2012). However, it was shown that only a subset of *S. epidermidis* strains express the *gseA* gene under the tested conditions (Iwase et al., 2010). This discrepancy shows that simple presence of a gene does not guarantee protein expression. Whether expression is induced in the presence of *S. aureus* still needs to be determined.

Similarly, *S. lugdunensis* inhibited both nasal and skin colonization of *S. aureus* via the production of **lugdunin**. Lugdunin is a thiazolidine-containing cyclic peptide antibiotic that is non-ribosomally synthesized and therefore belongs to a new class of bacteriocins. It displays potent antimicrobial activity against a wide range of Gram-positive bacteria including *S. aureus*. Interestingly, humans that carry *S. lugdunensis* have a six-fold lower risk of *S. aureus* nasal carriage due to lugdunin production (Zipperer et al., 2016). Notably, *S. aureus* did not manage to develop escape or resistance mechanisms neither to Esp (Iwase et al., 2010) nor to lugdunin (Zipperer et al., 2016) after multiple generations raising a strong interest in these naturally-derived antibiotics compared to conventional antibiotics in the treatment of *S. aureus* infections.

Moreover, *S. epidermidis* also produces **phenol-soluble modulins** (PSMy and PSMδ) which exert antimicrobial activity against *S. aureus* (Cogen et al., 2010).

Moreover, CoNS are able to control *S. aureus* colonization via **interference with** its **quorum sensing** signaling: In all staphylococci quorum sensing is achieved by the accessory gene regulator (*agr*) system. However, across staphylococci there is divergence within the *agr* locus resulting in different subtypes within and among species (Thoendel et al., 2011). In the following the *S. aureus agr* sytem type I is explained: Short autoinducing peptides (AIPs) are secreted by *S. aureus* and thereby the AIP density correlates with bacterial density. *S. aureus* senses the AIP concentration through the sensor histidine kinase AgrC which activates the downstream DNA-binding response regulator AgrA upon reaching a critical level of AIP. Subsequently, AgrA binds to the P2 promoter and induces the transcription of the agrBDCA operon resulting in a positive feedback regulation with transcription of the two-component system AgrCA as well as further AIP production and export through the exporter AgrBD. Moreover, AgrA binds to the P3 promoter and induces the transcription of RNAIII which is a global regulator of multiple *S. aureus* virulence factors (Le and Otto, 2015; Novick and Geisinger, 2008; Thoendel et al., 2011). It was shown that *S. epidermidis* can downregulate *S. aureus* virulence through quorum sensing interference (Otto et al., 2001; Otto et al., 1999). Screening for other CoNS that potentially inhibit transcription of the *S. aureus agr* operon, *S. caprae*, a bacterium that was only recently identified to colonize human skin (d'Ersu et al., 2016) was shown to potently inhibit *agr* and *RNAIII* transcription (Paharik et al., 2017). Furthermore, in an intradermal mouse skin injection model, *S. caprae* significantly reduced MRSA-induced tissue damage and bacterial burden by blocking *S. aureus* quorum sensing (Paharik et al., 2017).

5.3.2 Effect of microbiota on skin immunity

The finding that the microbiome largely influences skin immunity is nicely illustrated by the fact that germ-free mice display a defect in innate immunity (Goris et al., 1985). In the context of functional effect on skin immunity, *S. epidermidis* is the most studied commensal. Therefore, functional effects mostly of *S. epidermidis* on skin immunity are described in the following:

In undifferentiated keratinocytes secreted factors of *S. epidermidis* were shown to induce hBD2 and hBD3 expression in a TLR2-dependent manner (Lai et al., 2010). Later, the same group isolated a novel lipopeptide, named LP01, from *S. epidermidis* which induced AMP expression in keratinocytes and increased the capacity of keratinocyte cell lysates to kill *S. aureus* in a TLR2/CD36-p38-MAPK-dependent manner (Li et al., 2013). Moreover, our group showed that *S. epidermidis* can amplify the innate defense of keratinocytes against *S. aureus* (Wanke et al., 2011). Another *S. epidermidis*-derived lipopeptide (LP78), which has the same amino acid sequence as LP01 but possesses an N-terminal heneicosanoic acid group instead of a Lysine-bound one, was shown to inhibit TLR3-mediated skin inflammation by activating TLR2 and inducing β-catenin phosphorylation (Li et al., 2019). Similarly, it was previously shown that upon injury keratinocytes require TLR3 signaling to induce inflammation and that *S. epidermidis* LTA-mediated activation of TLR2 antagonized the release of pro-inflammatory cytokines (Lai et al., 2009). Microbiota can also limit TSLP expression in mice with a defective skin barrier and thus prevent allergic skin inflammation (Yockey et al., 2013).

A density of 1 million T cells per cm^2 of skin (Clark et al., 2006) indicates that there are also commensal-specific effects on adaptive skin immunity: In germ-free mice or mice treated with broad spectrum antibiotics T_H17 cell frequencies both in the skin and in the gut are severely reduced (Ivanov et al., 2008; Naik et al., 2012). In the gut mono-colonization with segmented filamentous bacteria restored the accumulation of T_H17 cells and was associated with protection against gastrointestinal pathogens (Ivanov et al., 2009). Similarly, germ-free mice which failed to produce IL-17A and IFNγ in αβT cells in the skin failed to control Leishmania major skin infections. Here, recolonization with *S. epidermidis* restored dermal IL-17A and IFNγ production and conferred protection against *L. major*. This development was shown to be independent from the gut microbiota but dependent on MyD88 and IL-1 signaling in the skin (Naik et al., 2012). Additionally, topical application of *S. epidermidis* onto mice was shown to induce IL-17A$^+$ CD8$^+$ T cell homing to the basal epidermis which enhances barrier immunity against *Candida albicans* infections by promoting IL-17A-mediated AMP production in interfollicular keratinocytes. They demonstrated that this commensal-driven CD8$^+$ T cell response is antigen-specific and is mediated by a subset of CD103$^+$ DCs by secretion of IL-1α. Whether this response also protects from *S. aureus* infection was not investigated (Naik et al.,

2015). Later, enrichment of these $CD8^+$ T cells was shown to be dependent on a specific clade (A20) of *S. epidermidis* which was previously shown to be highly prevalent on healthy human skin (Byrd et al., 2017; Linehan et al., 2018). Furthermore, *S. epidermidis*-specific $CD8^+$ T cells express genes associated with wound healing and tissue repair which promoted and accelerated wound closure in a skin punch biopsy wounding model (Linehan et al., 2018).

In addition to the group of Yasmine Belkaid, Scharschmidt et al. (2015) also reported an antigen-specific T cell response upon *S. epidermidis* skin colonization, which, however, led to immune tolerance: Colonization of neonatal mice with *S. epidermidis* resulted in antigen-specific T_{reg} accumulation in the skin which also prevented aberrant skin inflammation. Using their model T cell antigen epi2W they found increased numbers of 2W-specific $CD4^+$ T_{regs} both in the skin as well as in the skin-draining lymph nodes indicating that commensal antigens were recognized locally and systemically across an intact skin barrier (Scharschmidt et al., 2015). In their follow-up paper they analyzed the mechanisms that drive T_{reg} migration into the skin. Given the fact that T_{reg} accumulation and penetration of the hair shaft through the epidermis coincides around postnatal day 5 they hypothesized that the development of hair follicles, which represent an important niche for skin microbiota, drives T_{reg} migration to the skin. Indeed, they showed that keratinocytes at the hair follicle express CCL20 which recruits $CCR6^+$ T_{regs} into the skin (Scharschmidt et al., 2017).

Upon analysis of differential effects of *S. epidermidis* and *S. aureus* it was shown that monocyte-derived DC (moDC) exposure to the secretome of *S. epidermidis* resulted in high levels of IL-10 secretion whereas exposure to *S. aureus* secretome induced IFNγ secretion, suggesting that skin commensals can counteract the inflammatory stimulus of pathogens. Moreover, *S. aureus*-primed moDCs induced $CD4^+$ T cell proliferation whereas moDCs stimulated with *S. epidermidis* secretome did not (Laborel-Preneron et al., 2015). In line with these results, LTA derived from *S. epidermidis* or *S. aureus* elicited opposing cytokine profiles on murine DCs: While *S. epidermidis* elicited an IL-10-balanced immune profile, *S. aureus* induced high levels of IL-12p70 which resulted in IFNγ and IL-17C production by DC-primed $CD4^+$ T cells (Volz et al., 2018). In conclusion, *S. epidermidis,* as part of the skin microbiota, contributes to skin homeostasis by multiple different mechanisms ranging from

induction of AMP production to adaptive T cell responses. The protective mechanisms employed by *S. epidermidis* are illustrated in Figure 7.

In addition to *S. epidermidis,* there are also other bacteria that have been associated with beneficial effects on the skin: For example, treatment of human moDCs or primary keratinocytes with *Acinetobacter lwoffi* induced IL-10 production which reduced ovalbumin-induced allergic sensitization in an intradermal injection model (Fyhrquist et al., 2014). Moreover, in a placebo-controlled study lysate of the Gram-negative *Vitreoscilla filiformis*, which is home to thermal spring water, significantly reduced skin inflammation in AD patients. In a murine model of AD with NC/Nga mice the lysate of *V. filiformis* suppressed skin inflammation by inducing IL-10 production in DCs in a TLR2/MyD88-dependent manner which resulted in T_{reg} induction *in vitro* and suppression of effector T cell proliferation in skin draining lymph nodes (Volz et al., 2014). Similarly, treatment of moDCs with protein extract of *Aquaphilus dolomiae*, a Gram-negative bacterium isolated from Avene Spring water, induced IL-10 production and reduced *S. aureus* secretome-induced proliferation of $CD4^+$ T cells (Martin et al., 2016).

Moreover, microbiota where not only shown to control bacterial infections locally but also systemically and distally: Catabolism of tryptophan-containing dietary components to indole derivatives by commensals in the gut dampens the severity of inflammatory skin conditions (Di Meglio et al., 2014) through the aryl hydrocarbon receptor (Yu et al., 2018). In addition to that, commensal-derived peptidoglycan found in serum can also improve *S. aureus* killing by neutrophils (Clarke et al., 2010). Moreover, *S. epidermidis* was also shown to have beneficial effects on the development of ultraviolet-induced skin cancer (Nakatsuji et al., 2018). Apart from influencing skin immunity, a comparison of the skin transcriptomes of germ-free mice and SPF mice revealed that the microbiota also regulates epidermal differentiation (Meisel et al., 2018).

In conclusion, commensals exhibit diverse effects on the skin which we are only beginning to understand. However, the effects of microbiota were also shown to be context-dependent: For example, repeated topical exposure to *Corynebacterium accolens* or its membrane derivative mycolic acid resulted in the expansion of a defined subset of IL-17^+ $\gamma\delta$T cells which was non-inflammatory during homeostasis. In the context of a high fat diet, however, these T cells promoted skin inflammation

(Ridaura et al., 2018). Moreover, in addition to *S. aureus* also levels of *S. epidermidis* were shown to be increased during the flares of AD (Kong et al., 2012). But until today it is unclear whether *S. epidermidis* contributes to inflammation or whether its levels rise to prevent *S. aureus* from colonizing.

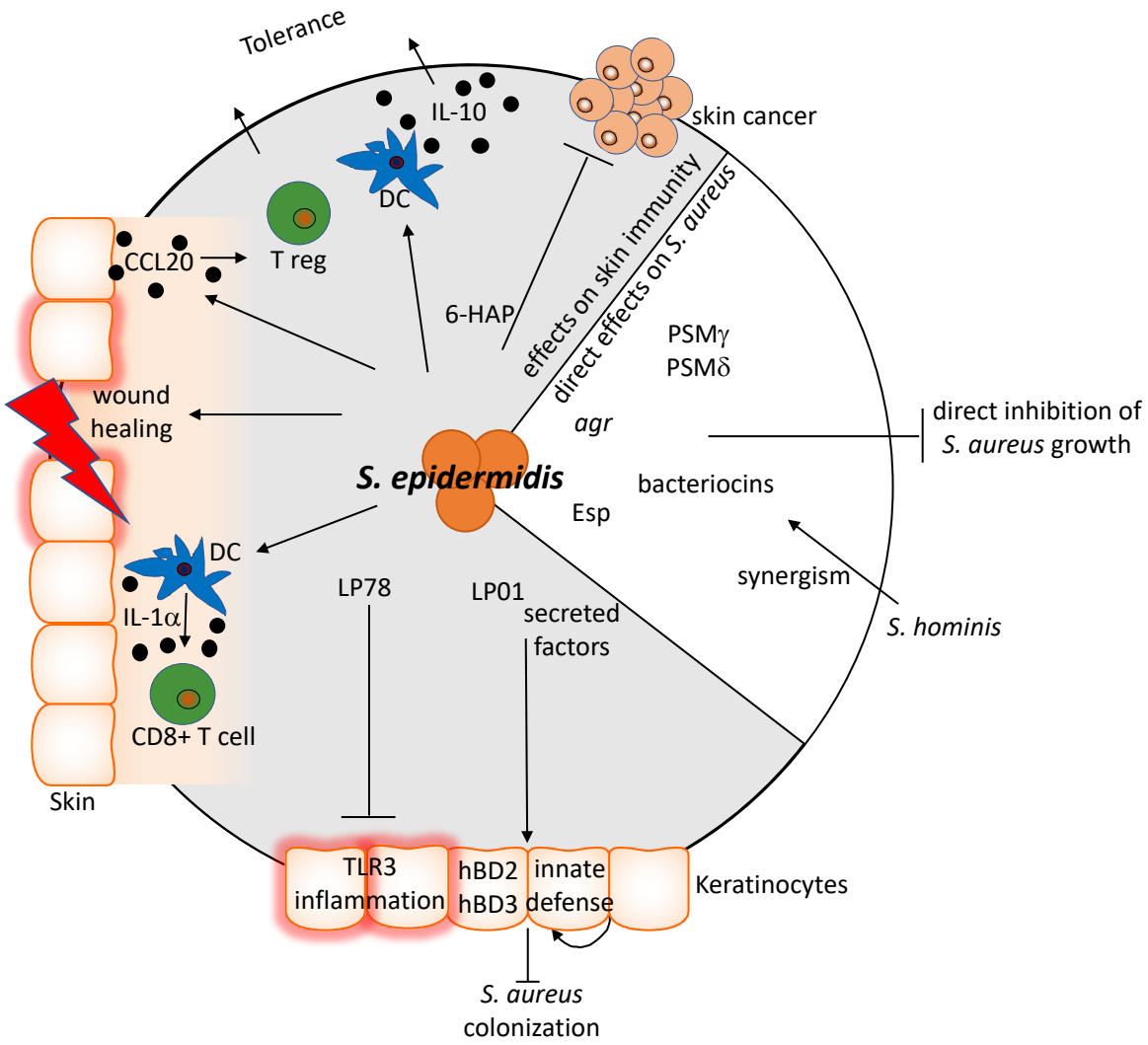


Figure 7: The miscellaneous functional effects of *S. epidermidis*

The commensal *S. epidermidis* exhibits diverse functional effects on host skin immunity: By directly targeting *S. aureus* it can shape pathogen skin colonization. Additionally, *S. epidermi*dis can interact with keratinocytes and other immune cells of the skin to counteract inflammation, infection, and cancer. Eclectic functional effects of *S. epidermidis* are shown in this figure. DC = dendritic cell; T reg = reulatory T cell; IL-10 = Interleukin-10; IL-1α = Interleukin-1α; PSM = Phenol soluble modulin; agr = accessory gene regulator; hBD = human beta defensin; TLR = Toll-like receptor; LP = Lipopeptide; 6-HAP = 6-*N*-hydroxyaminopurine.

5.3.3 Outlook: applications of skin microbiota

Conventional therapeutic strategies in AD have sought to target *S. aureus* by using broad-spectrum oral or topical antibiotics such as mupirocin as well as antiseptics. However, these strategies rarely prevent long-term *S. aureus* colonization and lack specificity. As a result, antibiotic treatment destroys the beneficial microbiota and at the same time selects for antibiotic-resistant *S. aureus* strains (Harkins et al., 2018a). In conclusion, current treatment options display a limited solution for patients suffering from staphylococcal super-infections and more targeted therapies are needed to combat *S. aureus* infections in AD patients. Since *S. aureus* frequently evolves antibiotic resistance (DeLeo et al., 2010) and vaccine development is nearly impossible (Jansen et al., 2013; Proctor, 2012) the idea of using microbiota to eradicate pathogens has been receiving a lot of attention. Microbiota can be harnessed to combat pathogen infections both by producing bacteriocins and by activating the immune system (Pamer, 2016). Promising strategies involve transplantation of microbiota from healthy donors to diseased which has already successfully been done in the gut in patients suffering from relapsing *Clostridium difficile* infections (van Nood et al., 2013). Therefore, unique antimicrobials or strains from healthy skin need to be identified and topically transplanted onto the skin of AD patients. Meylan et al. (2017) already show that presence of CoNS such as *S. hominis* during infancy can be protective against AD (Meylan et al., 2017). In line with this, many CoNS produce peptide antibiotics which specifically kill *S. aureus* and thus provide protection: *S. epidermidis* and *S. hominis* were shown to produce novel lantibiotics which were missing in AD patients. A single application of these lantibiotic-producing staphylococci onto the skin of AD patients significantly reduced the number of *S. aureus* colonizing the skin demonstrating the translational potential of this probiotic strategy (Nakatsuji et al., 2017). Similarly, 10 adult and 5 pediatric patients were enrolled in an open-label phase I/II safety and activity trial. Treatment with the commensal *Roseomonas mucosa* was associated with significant decreases in disease severity and *S. aureus* burden in AD patients (Myles et al., 2018).

Interestingly, a low diversity of the gut microbiome during the first month of life was associated with subsequent development of AD (Abrahamsson et al., 2012; Ismail et al., 2012; Wang et al., 2008). In line with this, probiotic supplementation of

the gut microbiome has beneficial effects on the development of AD (Foolad et al., 2013):

The adjuvant effect of the microbiota was not only shown during pathogen infection but also during cancer therapy: For example, cyclophosphamide, an anti-cancer immunomodulatory agent, leads to intestinal damage and concomitant microbial translocation which was shown to promote T_H17, T_H1 and $T_{\gamma\delta}$ cell responses that collectively contribute to the anti-tumor response (Daillere et al., 2016). In addition, upon disruption of the gut microbiota via antibiotic treatment or in germ-free mice myeloid-derived cells responded poorly to tumor therapy and the capacity of the host to control subcutaneous tumors was shown to be impaired (Iida et al., 2013). Moreover, immune checkpoint blockade of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) leads to inhibition of regulatory pathways in T cells and thus enhances anti-tumor activity dependent on distinct bacteroides species. Fecal transplant of human gut microbiota to mice confirmed that that treatment of melanoma with CTLA-4 antibodies favored the outgrowth of *B. fragilis* with anti-cancer properties (Vetizou et al., 2015). Furthermore, in mice bifidobacteria alone improved melanoma outgrowth to the same degree as PD-L1 antibody therapy and combination nearly abolished tumor growth in a dendritic cell $CD8^+$ T cell mediated manner (Sivan et al., 2015).

In conclusion, commensals control various aspects of immunity both locally and systemically. Uncovering the single pathways and mechanisms and identifying more commensal-derived molecules involved in these processes bears huge potential for the further development of commensal adjuvants that can be applied in the treatment of infectious diseases, autoimmune diseases as well as cancer.

6 Aim of this Thesis

Our skin is constantly exposed to a large number of pathogens, while at the same time commensal bacteria are ubiquitously colonizing. While CoNS are among the main colonizers, *S. aureus* is rarely found on healthy human skin. However, *S. aureus* is frequently isolated from the inflamed lesional skin of AD patients where it further causes skin inflammation and contributes to the worsening of the disease (Schittek, 2011). Thereby, overabundance of *S. aureus* during AD flares is associated with a loss of skin microbiome diversity indicating that skin commensal bacteria shape *S. aureus* skin colonization (Kong et al., 2012). Therefore, the overall aim of this thesis was to investigate the functional effects of two skin commensals, *S. epidermidis* and *S. lugdunensis,* on *S. aureus* skin colonization. Additionally, this work also aimed at understanding how skin inflammation contributes to initial *S. aureus* skin colonization.

The specific aims for the included publications were the following:

Accepted Publication I: The aim of this publication was to assess whether *S. epidermidis* protects primary human keratinocytes and mouse skin from *S. aureus* skin colonization both during skin homeostasis (i) and during skin inflammation (ii) induced by tape-stripping.

Accepted Publication II: The aim of this publication was to (i) assess whether *S. lugdunensis*-derived lugdunin induces skin protection similar to *S. epidermidis* and whether it affects the *S. epidermidis*-induced protective effect as well as to (ii) analyze whether lugdunin is able to induce an innate immune response in the skin. And finally, (iii) to investigate whether lugdunin can cooperate with other commensal- and host-derived AMPs to kill *S. aureus*.

Accepted Publication III: The aim of this publication was to evaluate (i) the diverse functional implications of keratinocytes in cutaneous *S. aureus* infection as well as (ii) their role as central players in microbial immune defense.

Accepted Manuscript I: The aim of this manuscript was to assess how skin inflammation increases *S. aureus* skin colonization. Therefore, the specific aims were to (i) compare the immune cell composition in healthy mouse skin to tape-stripped skin and to (ii) analyze the role of the resulting increased number of neutrophils in the

skin in *S. aureus* skin colonization. Additionally, (iii) the mechanism how neutrophils enhance *S. aureus* skin colonization needed to be investigated. Finally, (iv) it needed to be analyzed whether *S. epidermidis* modulates neutrophil recruitment in mouse skin.

7 Results and Discussion

Epithelial barrier tissues such as the skin are constantly exposed to an immense number of potential pathogens, while at the same time commensal bacteria are allowed to colonize. In this complex ecosystem CoNS are among the main colonizers (Grice and Segre, 2011). Thereby, *S. epidermidis* is the most frequently isolated skin commensal in humans (Grice and Segre, 2011; SanMiguel and Grice, 2015). *S. aureus,* however, can only rarely be found on healthy human skin, but was found to asymptomatically colonize the nose of 30% of the human population (Wertheim et al., 2005). Additionally, *S. aureus* is frequently isolated from the skin of AD patients where it further contributes to skin inflammation which consequently leads to disease worsening (Schittek, 2011). Interestingly, overabundance of cutaneous *S. aureus* especially during AD flares is associated with a loss of microbiome diversity indicating that the skin microbiome shapes *S. aureus* skin colonization (Kong et al., 2012).

In this work it was investigated how the two CoNS of our skin microbiota, *S. epidermidis* and *S. lugdunensis*, can protect the skin from *S. aureus* colonization during homeostasis (Accepted Publication I, II and Accepted Manuscript I). Additionally, it was investigated whether and how skin inflammation contributes to *S. aureus* colonization (Accepted Manuscript I) and how *S. epidermidis* shapes *S. aureus* colonization in an inflammatory environment (Accepted Publication I).

7.1 *S. epidermidis* protects the skin against *S. aureus* colonization

As the most frequently isolated skin commensal, *S. epidermidis* was shown to contribute to skin homeostasis and prevent pathogen colonization by multiple mechanisms (see section 5.3). For example, it has previously been shown that *S. epidermidis* can block *S. aureus* virulence factor expression by cross-inhibition of the *agr*-operon (Otto et al., 1999) or it can prevent *S. aureus* skin colonization by the production of a protective lipopeptide (Lai et al., 2010; Li et al., 2013) or by producing PSMs which can cooperate with host-derived AMPs to kill pathogens (Cogen et al., 2010). Additionally, it was previously shown by our group, that *S. epidermidis* is able to amplify the innate immune response of primary human keratinocytes (PHKs) (Wanke et al., 2011). However, the effect of *S. epidermidis* on *S. aureus* colonization on healthy versus inflamed skin has not been investigated. In our study we analyzed the influence of *S. epidermidis* on *S. aureus* skin colonization both *in vitro* and *in vivo* by using either PHKs or an epicutaneous mouse skin infection model with healthy skin or during skin inflammation induced by tape-stripping (Burian et al., 2017; Wanke et al., 2013; Zipperer et al., 2016).

In the *in vitro* infection model differentiated PHKs were pretreated with either living *S. epidermidis* 1457 (multiplicity of infection (MOI) = 30) for 2 hours or *S. epidermidis*-conditioned medium (CM) for 24 hours. Subsequently, cells were infected with the *S. aureus* strains USA300 or SA113 (MOI = 30) for 1.5 hours and the number of colony forming units (CFUs) that adhered to PHKs and invaded PHKs were analyzed. The data presented in Accepted Publication I, Figure 1 demonstrate that *S. epidermidis* significantly protects PHKs against *S. aureus* adhesion and invasion. Interestingly, this protective effect could be observed both by using live *S. epidermidis* and *S. epidermidis*-CM, indicating that *S. epidermidis*-secreted factors are responsible for immune conditioning of the epithelial barrier. Of note, usage of CM derived from other skin-derived and clinical *S. epidermidis* strains result in a similar reduction of *S. aureus* colonization. However, reduced *S. aureus* colonization of PHKs was neither observed upon pretreatment with *S. aureus* CM itself nor upon pretreatment with CM derived from other commensal Staphylococci. Of note, CM of *Corynebacterium pseudodiphteriticum* and *S. haemolyticus* induced a slight, however not significant, reduction in *S. aureus* colonization. In summary, these data indicate that the observed protective effect is a general effect that can be attributed to *S. epidermidis*.

To examine whether *S. epidermidis* also exerts this protective effect *in vivo* we used an epicutaneous mouse skin colonization model: Here, 15 µL of live *S. epidermidis* bacterial cells (10^9), *S. epidermidis* CM or PBS as a control was epicutaneously applied 24 hours before *S. aureus* USA300 (10^8) application on the shaved dorsal skin of C57BL/6 mice by using Finn Chambers® (Smart Practice). 24 hours after *S. aureus* application the number of surface-attached CFUs as well as of CFUs located in deeper skin tissue was determined. The data in Accepted Publication I, Figure 2 demonstrate that pretreatment of mouse skin both with live *S. epidermidis* and with *S. epidermidis* CM results in significantly lower *S. aureus* colonization compared to control mice indicating that also *in vivo S. epidermidis* and its secreted factors induce a protective mechanism which prevents *S. aureus* from colonizing.

Since *S. aureus* is frequently isolated from the inflamed skin of AD patients (Tauber et al., 2016; Totte et al., 2016; Travers et al., 2010) and we could already show that induction of skin inflammation by tape-stripping increases *S. aureus* colonization *in vivo* (Wanke et al., 2013) we proceeded to investigate whether *S. epidermidis* also exerts its protective capacities in an inflammatory environment *in vivo*. Therefore, we induced skin inflammation by repeatedly (7x) pulling off tape from the shaved dorsal skin of mice just before application of *S. epidermidis*, *S. epidermidis* CM or PBS. Interestingly, upon induction of skin inflammation the protective effect of *S. epidermidis* CM was lost whereas the effect was completely reversed upon pretreatment with live *S. epidermidis* which even significantly increased *S. aureus* skin colonization. Since this significant increase was only observed upon pretreatment with living *S. epidermidis* and not with CM these results indicate that here, a cell-bound compound of *S. epidermidis* is responsible for enhanced *S. aureus* colonization during skin inflammation.

To summarize, in Accepted Publication I we show that *S. epidermidis* as a member of the skin microbiota induces a protective effect on *S. aureus* skin colonization both *in vitro* on keratinocytes and *in vivo* during homeostasis. However, *S. epidermidis*-induced skin protection is dependent on the integrity of the epithelial barrier and is completely reversed by the induction of skin inflammation by tape-stripping. Considering previous data that show that *S. epidermidis* can amplify the innate immune response of human skin (Wanke et al., 2011) we conclude that skin

commensals create a protective environment by immune conditioning the healthy epithelial surface towards a protective immune response. However, skin inflammation itself can promote pathogen colonization and infection which might result in the suppression of the protective mechanism of skin commensals.

In the following work (section 7.2), it was analyzed whether the peptide antibiotic lugdunin derived from *S. lugdunensis*, another skin commensal, is equally well able to induce skin protection against *S. aureus* and whether lugdunin influences the *S. epidermidis*-induced protective effect. The work presented thereafter (section 7.3) aims at revealing the mechanisms how skin inflammation contributes to enhanced *S. aureus* colonization and presents a possible mechanism why *S. epidermidis* is unable to protect against *S. aureus* during skin inflammation.

7.2 Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors

S. lugdunensis is most frequently found in humans on the skin in the inguinal and perineal areas, the axilla, as well as in the nail bed and the nose (Bieber and Kahlmeter, 2010; Zipperer et al., 2016) and is considered a part of the normal human skin microbiota. *S. lugdunensis* can co-exist with other commensals on human skin such as *S. epidermidis* (Bieber and Kahlmeter, 2010; Gong et al., 2006), but intriguingly, nasal colonization by *S. aureus* or *S. lugdunensis* (Bieber and Kahlmeter, 2010; Zipperer et al., 2016) was shown to be mutually exclusive (Zipperer et al., 2016). In fact, humans who are colonized by *S. lugdunensis* in the nose have a 6-fold lower risk of *S. aureus* carriage than individuals who are not colonized (Zipperer et al., 2016). However, the total number of *S. lugdunensis* in the noses of carriers is considerably low compared to the absolute numbers of other commensals colonizing the nasal epithelium (Zipperer et al., 2016). Mutual exclusiveness was shown to result from the production of lugdunin, a novel peptide antibiotic produced by *S. lugdunensis* (Zipperer et al., 2016). While bacteria from the human microbiota have long been found to produce bacteriocins acting against closely related bacteria (Dobson et al., 2012; Kommineni et al., 2015), lugdunin represents the founding member of a new class of cyclic thiazolidine-containing peptide antibiotics (Zipperer et al., 2016). It exhibits high antimicrobial activity in the micromolar range against a wide range of Gram-positive bacteria including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* isolates and *Bacillus subtilis*.

7.2.1 Lugdunin amplifies *S. epidermidis*-induced skin protection against *S. aureus*

Similar to the protective effect induced by *S. epidermidis* presented in Accepted Publication I (Burian et al., 2017), our groups previously demonstrated that lugdunin effectively reduces *S. aureus* epithelial colonization in rodents and humans (Zipperer et al., 2016). In contrast to *S. epidermidis* application which was administered before *S. aureus* colonization, lugdunin was applied one and two days after *S. aureus* colonization in these experiments (Zipperer et al., 2016). Obviously, the direct bactericidal effect of lugdunin led to *S. aureus* killing. However, considering the fact that human nasal colonization with either *S. lugdunensis* or *S. aureus* was shown to

be mutually exclusive (Zipperer et al., 2016), even though absolute numbers of *S. lugdunensis* were considerably low, we investigated whether there might be an additional mechanism mediated by immune conditioning of epithelial cells by lugdunin. Therefore, we first tested whether pretreatment of PHKs or mouse skin with lugdunin also results in reduced *S. aureus* colonization by using the described models from Accepted Publication I (section 7.1). Indeed, pretreatment of PHKs with lugdunin alone at concentrations of 2 µM and above significantly reduced the number of adhering *S. aureus* (Accepted Publication II, Figure 1a) and concurrently, pretreatment of mouse skin with lugdunin alone resulted in a slight, however not significant, reduction of colonizing *S. aureus* (Accepted Publication II, Figure 1b). Strikingly, lugdunin-mediated reduction of *S. aureus* colonization was significantly enhanced both *in vivo* and *in vitro* upon combining lugdunin with *S. epidermidis* CM (Accepted Publication II, Figure 1a+b). In conclusion, these data indicate that lugdunin is not only able to directly kill *S. aureus* but can also sensitize keratinocytes towards a protective response against *S. aureus* skin colonization which can even be potentiated by secreted factors produced by *S. epidermidis*.

7.2.2 Lugdunin induces LL-37 and CXCL8 in keratinocytes

To elucidate the mechanism of the lugdunin-induced protective response, we analyzed whether lugdunin is able to induce the expression of AMPs or pro-inflammatory cytokines in PHKs, either alone or in combination with *S. epidermidis* CM. PHKs express a basal level of hBD-1, hBD-2 and hBD-3 as well as LL-37 and RNase7 (Schroder and Harder, 2006) and our groups previously demonstrated that *S. epidermidis* CM is able to induce hBD-1, hBD-2 and LL-37 expression (Wanke et al., 2011) which was confirmed in this work (Accepted Publication II, Figure 1c). Interestingly, lugdunin treatment of PHKs alone significantly induced LL-37 expression and release in a dose-dependent manner as well as expression of hBD-1 and RNase7 (Accepted Publication II, Figure 1c and Figure S1a+b). Surprisingly, combined treatment of PHKs with *S. epidermidis* CM and lugdunin did not amplify but abolished the effect of *S. epidermidis* CM on the induction of the respective AMPs, except for LL-37 (Accepted Publication II, Figure 1c). Next, we analyzed, whether lugdunin is able to induce the expression and secretion of different pro-inflammatory cytokines and chemokines in PHKs. Interestingly, increasing lugdunin-concentrations up to 3 µM specifically induced expression and release of CXCL8 in PHKs (Accepted

Publication II, Figure 1d+e and Figure S1c), while higher concentrations did not induce CXCL8 production (Accepted Publication II, Figure 1e and Figure S1c). Conversely, expression and release of LL-37 increased with higher lugdunin concentrations (Accepted Publication II, Figure S1a+b). Lugdunin-induced LL-37 and CXCL8 secretion was further confirmed by topical application of lugdunin on a human 3D skin equivalent (Accepted Publication II, Figure S1d). Additionally, in order to confirm that lugdunin induces LL-37 expression in keratinocytes also *in vivo* we performed immunohistochemical stainings of mouse skin sections upon epicutaneous application of lugdunin and *S. lugdunensis*. Indeed, keratinocytes in the epidermis and at the hair follicles, which were also the locations of *S. lugdunensis* colonization (Accepted Publication II, Figure S2d), showed a positive LL-37 staining (Accepted Publication II, Figure S1e).

In order to exclude potential lipopeptide contamination of purified lugdunin (Hashimoto et al., 2006) we compared CXCL8 induction in PHKs by synthetic and natural lugdunin. However, treatment of PHKs both with synthetic and natural lugdunin resulted in similar CXCL8 expression levels. In fact, the potential of lugdunin to induce CXCL8 seems to be dependent on its direct antimicrobial activity since the non-antimicrobial N-Acetyl-lugdunin (Schilling et al., 2019) did not induce CXCL8 expression in PHKs (Accepted Publication II, Figure S1f). In conclusion, antimicrobial activity of lugdunin with its original thiazolidine heterocycle building block seems to be crucial for innate immune activation in keratinocytes.

Notably, lugdunin was also able to induce CXCL8 expression in primary human nasal and tracheal epithelial cells (HNEpCs and HTEpCs) as well as in peripheral blood mononuclear cells (PBMCs) (Accepted Publication II, Figure S1g-i), however, with a much lower efficacy compared to PHKs. Of note, basal protein levels of CXCL8 in these cells were already higher compared to PHKs and could not be further increased by lugdunin treatment (Accepted Publication II, Figure 1f). In HNEpCs and HTEpCs, but not in PHKs and PBMCs, lugdunin was also able to induce the release of IL-1α (Accepted Publication II, Figure 1d+g), another important pro-inflammatory cytokine involved in epithelial barrier immunity (Naik et al., 2015; Naik et al., 2012). Of note, the observed induction of pro-inflammatory cytokines in the different cell types is not a result of possible cytotoxic features of lugdunin since it did not display any cytotoxicity on the cell types used in this study (Accepted

Publication II, Figure S1j) nor on primary human neutrophils, erythrocytes or the human monocytic cell line HL60 (Zipperer et al., 2016).

In summary, our data indicate that lugdunin is able to induce expression of LL-37 and pro-inflammatory cytokines in PHKs which might modulate the response of PHKs towards *S. aureus* skin colonization. Additionally, the type of immune response induced by lugdunin is cell-type specific, which might correlate with the different sites of *S. lugdunensis* colonization observed in humans (Bieber and Kahlmeter, 2010).

7.2.3 Lugdunin-induced cytokine production is TLR/MyD88-dependent

Since CXCL8 expression can be induced in PHKs and other cell types by activation of TLR2 (Fournier, 2012) we analyzed the potential role of TLR2 in lugdunin-induced CXCL8 release by using HEK293-TLR2 cells, which were transfected with a TLR2 containing plasmid leading to surface expression of TLR2 as well as HEK293 cells as a control, which do not express TLR2 (Hanzelmann et al., 2016). Pam2Cys and Pam3Cys positive control treatments of HEK293-TLR2 cells resulted in increased CXCL8 expression and release in a TLR2-dependent way (Accepted Publication II, Figure S2). Indeed, also lugdunin resulted in CXCL8 expression and release in a TLR2-dependent fashion, however, induction levels were 1000 and 10-fold lower for protein and expression levels respectively compared to positive control treatments suggesting that lugdunin might be a weak TLR2 agonist or it might induce CXCL8 expression by an indirect TLR2 activating mechanism (Accepted Publication II, Figure 2a, b, Figure S2a+b). The latter could resemble the mechanism of staphylococcal PSMs which were shown to mobilize TLR2-activating lipopeptides from bacterial membranes (Hanzelmann et al., 2016).

To further investigate the involvement of TLRs, we analyzed whether lugdunin is able to induce similar cytokines in murine skin as in PHKs. Therefore, we epicutaneously treated mouse skin with lugdunin or PBS as a control for 24 hours and determined the levels of pro-inflammatory cytokines in the skin (Accepted Publication II, Figure 2c-e, Figure S2c). Interestingly, we found that specifically IL-1α and MIP-2, the functional murine homologue of human CXCL8 (Zlotnik and Yoshie, 2000), were induced by lugdunin (Accepted Publication II, Figure 2d+e). Levels of other cytokines such as MCP-1, GM-CSF, TNFα, IL-6, and IFNγ did not show significant differences compared to the control group except for the anti-inflammatory

cytokine IL-10 (Accepted Publication II, Figure S2c). Similar to LL-37 (Accepted Publication II, Figure S1e), immunohistochemical analyses of mouse skin sections confirmed that keratinocytes in the epidermis and at the hair follicles, the primary sites for *S. lugdunensis* colonization (Accepted Publication II, Figure S2d) produce MIP-2 (Accepted Publication II, Figure 2f).

To analyze the importance of TLR- and MyD88-signaling in lugdunin-mediated cytokine induction, we additionally analyzed cytokine levels upon lugdunin treatment in the skin of mice deficient for MyD88 (MyD88-ko) or for TLR2, TLR3, TLR4, TLR7 and TLR9 (5xTLR-ko). However, expression of most cytokines was not significantly different upon lugdunin treatment compared to WT control mice (Accepted Publication II, Figure S2c). Lugdunin was still able to induce IL-1α in the skin of MyD88-ko and 5xTLR-ko mice (Accepted Publication II, Figure 2d). Lugdunin-induced MIP-2 induction, however, was completely impaired in mice lacking MyD88 and reduced in 5xTLR-ko mice (Accepted Publication II, Figure 2e) thus confirming the *in vitro* data. In conclusion, these data show that lugdunin induces CXCL8/MIP-2 in keratinocytes by a TLR/MyD88-dependent mechanism.

7.2.4 Epicutaneous lugdunin recruits phagocytic cells

Keratinocytes express several pattern recognition receptors such as TLR2, which recognizes *S. aureus* lipopeptides (Ryu et al., 2014). Activation of TLR2 leads to MyD88-dependent activation of NF-κB and other transcription factors, which subsequently induce the transcription of pro-inflammatory chemokines and cytokines such as CXCL8 and IL-1α as well as AMPs involved in cutaneous host defense against *S. aureus* (Ryu et al., 2014). While IL-1α was already shown to be induced in the skin by commensals where it substantially contributes to skin immunity (Naik et al., 2015; Naik et al., 2012), induction of CXCL8/MIP-2 expression in keratinocytes is an immediate and early pro-inflammatory response resulting in the recruitment of phagocytic immune cells to clear infections (Krishna and Miller, 2012; Miller and Modlin, 2007; Rot and von Andrian, 2004). Therefore, we analyzed the composition as well as the potential recruitment of immune cells into the skin of C57BL/6 WT, MyD88-ko and 5xTLR-ko mice 24 hours after epicutaneous treatment with lugdunin or PBS (Accepted Publication II, Figure 3a-d and Figure S3). In line with the lugdunin-induced MIP-2 induction, we found significantly enhanced recruitment of monocytes and neutrophils in the skin of mice treated with lugdunin which was

completely impaired in MyD88-ko and 5xTLR-ko mice (Accepted Publication II, Figure 3d). Additionally, we performed immunohistochemical staining of myeloperoxidase (MPO) in mouse skin sections demonstrating that epicutaneous application of both lugdunin and the lugdunin-producing *S. lugdunensis* results in recruitment of MPO-positive cells into the dermis (Accepted Publication II, Figure 3e).

Percentages of live $CD45^+$ cells (Accepted Publication II, Figure 3b), B, T, and NK cells as well as total $CD11b^+$ cells and dendritic cells did not substantially differ from the PBS control upon treatment (Accepted Publication II, Figure 3c and Figure S3). Interestingly, macrophage levels were slightly reduced in percentage upon lugdunin treatment (Accepted Publication II, Figure 3c + Figure S3). Taken together, these results indicate that CXCL8/MIP-2 induction by lugdunin in mouse skin and in PHKs is mediated by a TLR/MyD88-dependent pathway in keratinocytes which leads to the recruitment of phagocytic innate immune cells such as monocytes and neutrophils. To conclude, clearance of pathogens by lugdunin-mediated recruitment of phagocytic cells complements its direct antimicrobial effects and thus provides an additional level of pathogen protection.

7.2.5 Lugdunin amplifies innate immune response of keratinocytes

Besides their bactericidal activity, host-derived AMPs have been shown to play a role in modulation of the innate immune defense (Schittek et al., 2008) and their expression was shown to be dysregulated in AD patients (Harder et al., 2010; Rieg et al., 2005; Schittek, 2011; Schittek et al., 2008; Yamasaki and Gallo, 2008). Since we showed that lugdunin is a very potent inducer of CXCL8 and LL-37 in PHKs, we asked whether other bacteria- and skin-derived AMPs are equally well able to induce CXCL8 expression in PHKs. We tested the bacteriocins nisin, gallidermin as well as the non-bactericidal pro-form pro-gallidermin and the human AMPs LL-37 and the dermcidin-derived peptides DCD-1 and DCD-1L. However, compared to the other AMPs and bacteriocins, lugdunin was especially potent in inducing CXCL8 expression in PHKs leading to over 40-fold induction (Accepted Publication II, Figure 4a). Only DCD-1 treatment resulted in a 10-fold induction of CXCL8 expression in PHKs. LL-37, nisin and (pro)-gallidermin, however, were not able to induce CXCL8 expression in PHKs.

Since we have already shown that skin protection against *S. aureus* is amplified when lugdunin is combined with *S. epidermidis* CM, we next investigated the potential of lugdunin and the other peptides to induce CXCL8 when combined with *S. epidermidis* CM. *S. epidermidis* CM alone was equally well able to induce CXCL8 expression in PHKs as lugdunin. Interestingly, CXCL8 induction could be highly amplified by the combination of *S. epid*ermidis CM and lugdunin from 40-fold to over 1000-fold (Accepted Publication II, Figure 4a). This effect was specific for lugdunin since all other peptides did not significantly affect *S. epidermidis*-induced upregulation of CXCL8 expression in PHKs. Noticeable, gallidermin completely blocked *S. epidermidis* CM-induced CXCL8 expression in PHKs (Accepted Publication II, Figure 4a). This observation is in line with recent data from Bengtsson et al. (2018) who found that gallidermin abolishes Staphylococci-induced release of CXCL8 and IL-6 in primary human dermal fibroblasts by which it may counteract a potentially harmful inflammatory process (Bengtsson et al., 2018).

Since lugdunin induced the expression of LL-37 in PHKs (Accepted Publication II, Figure 1c), we additionally investigated CXCL8 expression in PHKs upon combinational treatments of lugdunin and the other bacterial antimicrobials with the human AMPs LL-37 or DCD-1L and DCD-1. Pam2Cys, IL-1α, or *S. epidermidis* CM treatment served as positive controls. As shown already in Accepted Publication II, Figure 4a, lugdunin treatment alone was already very potent in inducing CXCL8 expression in PHKs which could not be further amplified by combined treatments (Accepted Publication II, Figure 4b).

Furthermore, DCD-1 single treatments as well as combinations that involved DCD-1 or DCD-1L significantly induced CXCL8 expression. These results are in agreement with Niyonsaba et al. (2009) who showed that DCD-1L activates NF-κB signaling in human keratinocytes and leads to the release of TNFα, CXCL8, interferon-inducible protein 10 (CXCL10) and macrophage inflammatory protein-3α (CCL20) (Niyonsaba et al., 2009b). Additionally, it was shown that dermcidin treatment of PBMCs induces the release of TNFα (Wang et al., 2016). DCD peptides are secreted by eccrine sweat glands where they can be transported to the skin surface and serve as a constant antimicrobial shield and are thus constitutively present on human skin (Rieg et al., 2004; Schittek et al., 2001) (Accepted Publication II, Table 1). Thus, being constantly present on the skin surface, our results suggest

that dermcidin peptides are able to increase the innate immune defense on the skin depending on the available commensal- or pathogen-derived peptide antibiotics. These data indicate that lugdunin is a very potent immune modulator of the skin that acts in concert with other microbiota-derived modulating factors.

In order to exclude that CXCL8 induction results from cytotoxicity of the used peptides we complement our previous results that showed that lugdunin does not display cytotoxicity on host cells (Accepted Publication II, Figure S1j) by demonstrating that none of the used peptides in this study show host cell cytotoxicity (Accepted Publication II, Figure S4). These results conform to data reported by other groups that showed that lantibiotics usually display low cell cytotoxicity (Maher and McClean, 2006).

7.2.6 Lugdunin acts synergistically with host-derived AMPs

In addition to its immune modulating activity lugdunin was discovered for its ability to directly kill *S. aureus* (Zipperer et al., 2016). Regarding its mode of action, it was shown that bacterial cells exposed to lugdunin stopped incorporating DNA, RNA, protein or cell-wall precursors even at concentrations below the minimal inhibitory concentration (MIC) suggesting that lugdunin may lead to a rapid breakdown of bacterial energy resources (Zipperer et al., 2016). Recently it was shown that lugdunin equalizes the bacterial membrane potential by proton translocation (Schilling et al., 2019). Considering this antimicrobial activity as well as its ability to induce LL-37 and host defense signaling via TLRs/MyD88 in skin we speculated that lugdunin might potentially act in concert with other antimicrobial substances to increase *S. aureus* killing. In the following, we investigated the capacity of lugdunin, (pro)-gallidermin and nisin to kill *S. aureus* in cooperation with LL-37 and DCD-1 and DCD-1L.

At first, we determined killing curves of the different human and bacterial AMPs for *S. aureus* USA300 in order to choose sub-bactericidal concentrations for subsequent synergistic studies (Accepted Publication II, Figure S5). Generally, in our experimental setting the efficiency of bacteriocins in *S. aureus* killing was higher than of the human AMPs. Although this work did not demonstrate a bactericidal effect of human AMPs on *S. aureus* at concentrations ranging from 1-15 µM, LL-37 has been described to inhibit *S. aureus* growth and kill *S. aureus* at 128 µg/mL (Niemirowicz et

al., 2016) and 19.3 µg/mL (Luo et al., 2017). This discrepancy to our data might result from using different bacterial strains: Usage of the highly virulent USA300 strain which expresses virulence factors such as the *mprf* and *dlt* operon, both of which were shown to change the electric charge of the bacterial cell wall resulting in rejection of the positively charged LL-37, might impact susceptibility to LL-37. Moreover, exposure of *S. aureus* to different cationic AMPs was shown to induce further upregulation of *mprf* or *dlt* (Otto, 2010; Ryu et al., 2014). Additional described resistance mechanisms of *S. aureus* are extracellular degradation of LL-37 via the proteases aureolysin and V8 protease and the AMP sensor system Aps, activation of which leads to changes in the bacterial surface charge via lysinylation of bacterial membrane lipids and D-alanylation of teichoic acids in the staphylococcal cell wall (Otto, 2009a; Ryu et al., 2014). Growth and viability of *S. aureus* were neither reduced by DCD-1(L) treatment at concentrations ranging from 1 µM to 15 µM. These results are supported by (Lai et al., 2007) describing a dermcidin concentration of around 10 µM as a sub-inhibitory concentration, whereas (Vuong et al., 2004) described an LD50 value of 5 µM. Nevertheless, it has to be noted that the antimicrobial activity of AMPs was shown to vary depending on the assay conditions. Human AMPs were shown to be less active against Staphylococci in the presence of trypticase soy broth medium which was used in our experimental setup (Schittek et al., 2001; Schwab et al., 1999). In medium resembling sweat dermcidin peptides were shown to exhibit antimicrobial activity against a broad spectrum of microorganisms including Staphylococci (Rieg et al., 2004; Vuong et al., 2004). Interestingly, in our experiments treatment with human AMPs increased *S. aureus* growth indicating the utilization of these peptides as nutrients.

For our synergy studies we chose concentrations of 1-3 µM of lugdunin, human AMP concentrations of 1 and 2 µM and (pro-)gallidermin and nisin concentrations ranging from 0.4-0.8 µM. Combinations of the human AMPs DCD-1(L) and LL-37 with sub-bactericidal concentrations of lugdunin or gallidermin and nisin resulted in enhanced *S. aureus* killing compared to the single treatments (Accepted Publication II, Figure 5a-c and Figure S6). Of note, the effect was specific for the active form of gallidermin since co-incubation with pro-gallidermin did not lead to antimicrobial activity (Accepted Publication II, Figure S5+6). Using CompuSyn software we analyzed potential synergistic effects of peptide combinations and calculated the combination indices for the indicated combinations. The activities of lugdunin and

gallidermin in combination with the human AMPs reached CI values below 1 indicating synergistic activity of these peptides (Figure 5b).

While expression of LL-37 can be increased by lugdunin in keratinocytes (Accepted Publication II, Figure S1a+b), dermcidin peptides are constantly present on human skin. Therefore, this work suggests that the presence of lugdunin-producing *S. lugdunensis* on the skin locally increases LL-37 expression but also acts in cooperation with constitutively expressed host-derived AMPs. Interestingly, it was recently shown that during steady-state, in the absence of pathogens, commensals do not express bacteriocins (Ebner et al., 2018). Similarly, lugdunin expression is induced only upon direct contact of *S. lugdunensis* and *S. aureus* (Zipperer et al., 2016). Thus, synergistic action of host- and bacteria-derived peptides can be employed when needed.

To analyze whether the synergistic activity is a specific effect on *S. aureus*, we tested the spectrum of antimicrobial activity of lugdunin alone or in combination with DCD-1(L). Accepted Publication II, Figure S7 shows that the combined effect of lugdunin with DCD-1(L) could neither be observed for the Gram-positive skin commensal *S. epidermidis* or intestinal *Enterococcus faecalis* nor for the Gram-negative bacteria *Pseudomonas aeruginosa, Escherichia coli*, or *Proteus mirabilis*. Of note, *Bacillus subtilis* was the only bacterial species tested besides *S. aureus* that was also susceptible to lugdunin and lugdunin/DCD-1(L) combinations (Accepted Publication II, Figure S7f).

Additionally, we investigated whether the synergistic activity relies on the combined action of the peptides or whether one peptide is able to sensitize for bacterial killing by the other peptide. Therefore, we performed sequential incubation steps of the single peptides (Accepted Publication II, Figure 6). Single as well as simultaneous combination treatments were always included as controls. However, sequential treatment of *S. aureus* with the synergistically active peptide concentrations did not lead to *S. aureus* killing (Accepted Publication II, Figure 6). From these data we conclude that there is a synergistic activity of host- and bacteria-derived peptides in *S. aureus* killing and that this synergistic effect seems to be dependent on simultaneous action of the bioactive peptides indicating that either pre-formed complexes of the peptides are synergistically active or that a dual mode of action with both peptides targeting different cellular structures leads to synergistic *S.*

aureus killing. Generally, such synergism of host- and commensal-peptides has rarely been reported: It has been shown that combination of the *S. epidermidis* Esp protease with hBD2 resulted in synergistic bactericidal activity that killed *S. aureus* in biofilms (Sugimoto et al., 2013). Moreover, the group of Richard Gallo recently demonstrated that AD patients are lacking bacteriocin-producing skin commensals and that re-establishment of bacteriocin-producing *S. epidermidis* and *S. hominis* prevents *S. aureus* from colonizing the skin of these patients. In this work they identified novel skin commensal-derived bacteriocins that act synergistically with LL-37. Additionally, a single application of these bacteriocin-producing strains significantly reduced *S. aureus* loads on the forearms of AD patients already after 24 hours indicating the great clinical potential of host and commensal peptide combinations (Nakatsuji et al., 2017). However, it is completely unknown whether other peptide antibiotics are equally well able to induce the expression of synergistically acting AMPs in skin as lugdunin. Considering their low cytotoxicity reported in Accepted Publication II, Figure S4 and their high antimicrobial potential, the synergistic peptide combinations described in this work might be ideal candidates for potential clinical applications in the topical treatment of *S. aureus* infection.

7.2.7 Conclusion

The results of this study show that lugdunin provides multi-level protection of the host against *S. aureus* (Figure 8). First, it can directly kill *S. aureus* and also act synergistically with the human AMPs DCD-1(L) and LL-37 in killing *S. aureus*. On a second level of protection, lugdunin amplifies the commensal-induced innate immune response in PHKs. And ultimately, lugdunin-induced recruitment of phagocytic cells might additionally contribute to effective eradication of *S. aureus*. It still has to be determined how lugdunin production is regulated and whether factors from the host side are able to induce lugdunin production by *S. lugdunensis.* Since applying bacteriocins or bacteriocin-producing bacteria onto the skin have already become valuable strategies for preventing *S. aureus* colonization (Nakatsuji et al., 2017), future therapies using the synergistic effect of AMPs and lugdunin as well as the lugdunin-induced enhancement of integral pathways of the cutaneous innate immune defense could be promising alternatives to simple antibiotic treatment. Especially the lack of resistance mechanisms of *S. aureus* against lugdunin demonstrates its high potential for clinical applications (Zipperer et al., 2016). And in the unlikely event of

resistance development, only its direct bactericidal effect is impaired, whereas its immune-modulating potential would remain unaffected.

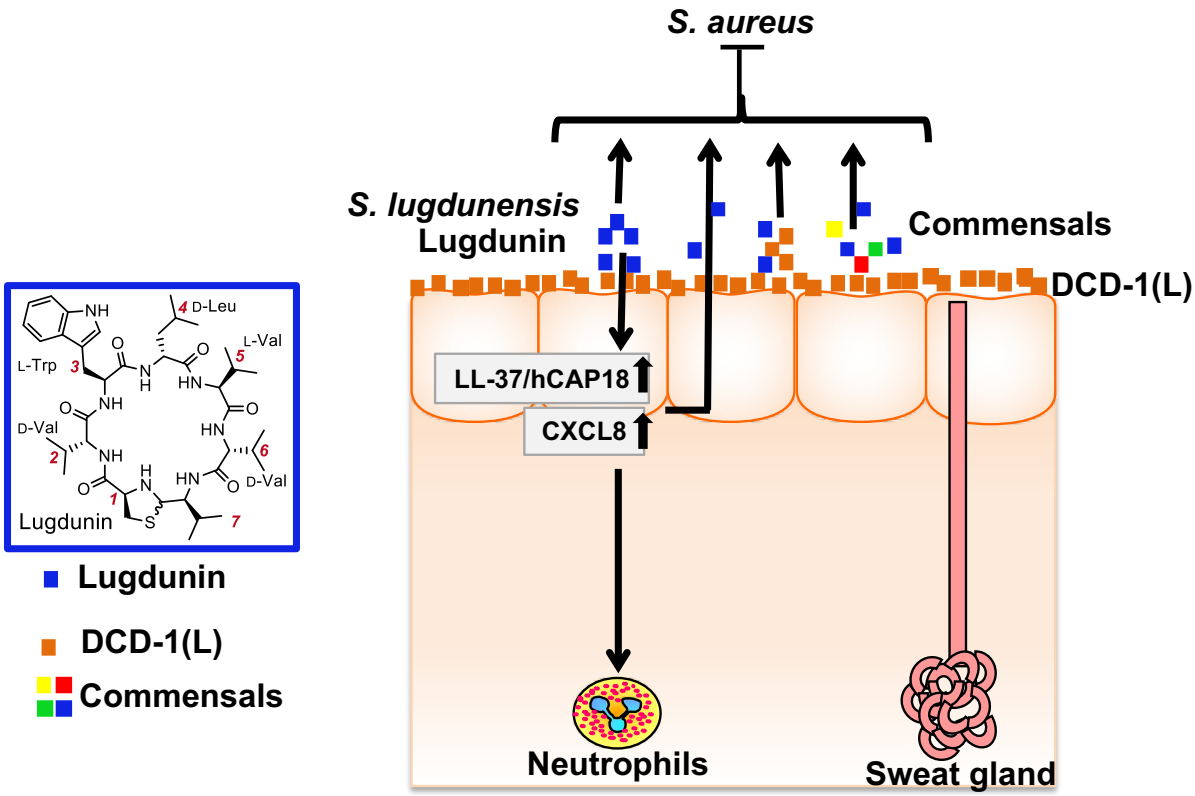


Figure 8: The different levels of lugdunin-mediated skin protection (Accepted Publication II, (Bitschar et al., 2019)

Lugdunin acts on multiple levels to protect the skin against an *S. aureus* infection: Firstly, lugdunin directly inhibits and kills *S. aureus*. Secondly, it cooperates with host-derived AMPs such as hCAP18/LL-37 as well as DCD-1(L) to synergistically kill *S. aureus*. Ultimately, by inducing an innate immune response in the skin leading to the recruitment of phagocytic immune cells that potentially clear invading pathogens, lugdunin provides a third level of skin protection. Additionally, this innate immune response can be highly amplified by *S. epidermidis*-derived factors.

7.3 *Staphylococcus aureus* skin colonization is enhanced by the interaction of neutrophil extracellular traps with keratinocytes

As previously shown in section 7.1 (Accepted Publication I (Burian et al., 2017)), *S. epidermidis* does not effectuate skin protection during skin inflammation. Additionally, it was previously demonstrated by our group that both *S. aureus* colonization and persistence on murine skin are significantly increased upon tape-stripping (Wanke et al., 2013). Therefore, this work aimed at analyzing the mechanisms that lead to enhanced *S. aureus* colonization in an inflammatory environment.

7.3.1 Skin inflammation induced by tape-stripping promotes *S. aureus* colonization *in vivo*

At first, we reproduced the data that show that *S. aureus* skin colonization increases in an inflammatory environment (Wanke et al., 2013) by comparing the colonization efficiency of healthy and tape-stripped mouse skin. By using two different *S. aureus* strains, USA300 and SA113, we could confirm that *S. aureus* colonization is enhanced upon tape-stripping (Accepted Manuscript I, Figure 1a). Interestingly, both the MRSA strain USA300 and the non-virulent and *agr*-deficient SA113 strain showed enhanced colonization upon tape-stripping already indicating that the effect might be independent of virulence factors which are mainly controlled by the *agr* quorum sensing system (see section 5.2.3.1).

Further analysis of the levels of pro-inflammatory cytokines in mouse skin 24 hours after tape-stripping showed that levels of TNFα, GM-CSF and IL-6 were clearly, however not significantly, induced whereas reduced levels of IL-10 could be observed (Accepted Manuscript I, Figure 1b). Levels of IL-1α, MIP-2, MCP-1 and IFN-β were not altered substantially (Accepted Manuscript I, Figure S1a). These results confirm previous work from others and our group demonstrating that tape-stripping leads to enhanced *S. aureus* colonization and persistence (Wanke et al., 2013) as well as pro-inflammatory cytokine expression in mouse (Malhotra et al., 2016; Onoue et al., 2009; Oyoshi et al., 2010; Wanke et al., 2013) and human skin (Dickel et al., 2010). Even though we could not detect changes in IL-1α levels, significant increases of IL-1α released from a pre-formed pool by keratinocytes in murine skin were reported within minutes upon tape-stripping indicating that IL-1α

might be an early orchestrator of skin inflammation upon tape-stripping (Wood et al., 1996).

In order to analyze whether an elevated pro-inflammatory environment induces enhanced *S. aureus* colonization, PHKs were treated with a cocktail of pro-inflammatory cytokines containing 10 ng/mL IL-1α, TNFα, Oncostatin M, IL-22 and IL-17A for 3 hours. This cytokine cocktail induced a massive pro-inflammatory response in PHKs resulting in strong expression of *IL1A*, *IL1B*, *TNFA, CXCL8* and *IL6* (Accepted Manuscript I, Figure S1b), however, in our established adhesion and invasion assay (see section 7.1) these cytokines did not affect *S. aureus* colonization efficiency (Accepted Manuscript I, Figure 1c). These data led us to assume that enhanced *S. aureus* colonization upon tape-stripping is not a direct effect of elevated pro-inflammatory cytokines but that there are other factors present *in vivo* that contribute to *S. aureus* colonization.

Therefore, we analyzed the composition of immune cell infiltrates in mouse skin 24 hours after tape-stripping (Accepted Manuscript I, Figure 2 and Figure S2). We found that only percentages of neutrophils (live CD45+ CD11b+ Ly6G+ Ly6C+) and monocytes (live CD45+ CD11b+ Ly6C+) are significantly increased (Accepted Manuscript I, Figure 2a and Figure S2) upon tape-stripping, while percentages of overall $CD45^+$ and other immune cell subsets are not significantly altered (Accepted Manuscript I, Figure S2). Further immunohistochemical staining for MPO-positive cells in mouse skin sections confirmed the enhanced recruitment of neutrophils (Accepted Manuscript I, Figure 2b). These results are in line with data observed by other groups who also show that tape-stripping induces early neutrophil recruitment within 1 day upon tape-stripping (Guiducci et al., 2010; Karisola et al., 2019; Takahashi et al., 2013).

The enhanced *S. aureus* skin colonization observed in this study resembles the elevated prevalence of *S. aureus* in patients suffering from AD (Leyden et al., 1974; Totte et al., 2016). AD is a multi-factorial disease with many factors such as genetic or environmental influences contributing to its pathogenesis (Eyerich et al., 2015) (see section 5.1.1). However, until today the mechanisms that promote *S. aureus* colonization during the onset of the disease as well as the role of the neutrophils during this process are unclear. Choy et al. (2012) compared the transcriptomic profile of AD skin to healthy skin and found that genes encoding for neutrophil

chemoattractants such as CXCL1, CXCL2, CXCL8 and GM-CSF as well as the neutrophilic infiltrate in the dermis were significantly elevated in AD skin compared to healthy controls (Choy et al., 2012). In line with this, Dhingra et al. (2013) compared neutrophil-related genes in AD skin to healthy skin and also found that neutrophil-related genes such as DEFB4A, CXCL8 and CXCL1 are significantly elevated in AD patients. Interestingly, they also associate higher neutrophil levels in AD with *S. aureus* infection (Dhingra et al., 2013). Taken together our data and the data presented in the above-mentioned literature suggest that an enhanced presence of neutrophils in the skin of AD patients contributes to the increased presence of *S. aureus* colonization and persistence on AD skin.

7.3.2 Presence of neutrophils in the skin increases *S. aureus* colonization

In order to test this hypothesis, we next investigated whether the presence of neutrophils in the skin contributes to elevated *S. aureus* colonization. Accordingly, we depleted neutrophils in C57BL/6 wild-type mice by injecting an anti-Ly6G antibody 24 hours prior to skin colonization with *S. aureus* USA300 (Accepted Manuscript I, Figure 3a). Indeed, compared to the IgG isotype control group, neutrophil depletion resulted in a significant reduction of *S. aureus* skin colonization in most anti-Ly6G-treated mice (Accepted Manuscript I, Figure 3b). These data clearly show that neutrophils contribute to enhanced *S. aureus* skin colonization. However, it should be noted that even though neutrophils were efficiently depleted, a small subgroup of mice inexplicably did not show reduced levels of *S. aureus*.

Since neutrophil recruitment in response to *S. aureus* skin infection was shown to be dependent on IL-1β and the IL-1R1 (Miller and Modlin, 2007; Miller et al., 2006) we subsequently used IL-1R1-ko mice as a model for defective neutrophil recruitment. Indeed, upon *S. aureus* skin colonization IL-1R1-deficient mice fail to recruit neutrophils. Notably, compared to WT mice, IL-1R1-ko mice already displayed lower basal numbers of neutrophils during homeostasis (Accepted Manuscript I, Figure 3c). In line with the reduced *S. aureus* colonization observed upon neutrophil depletion, colonization of IL-1R1-ko mice was significantly lower compared to WT mice (Accepted Manuscript I, Figure 3d).

On a first glance the fact that neutrophils contribute to enhanced *S. aureus* skin colonization may seem bizarre since neutrophil migration to infected tissues and

concomitant pathogen clearance is a fundamental component of innate immunity (Kennedy and DeLeo, 2009; Verdrengh and Tarkowski, 1997). Furthermore, it has previously been demonstrated by other groups that upon *S. aureus* skin infection recruitment of neutrophils is critical in infection resolution (Miller et al., 2006; Molne et al., 2000). Yet on a closer look, these data rely on subcutaneous or intradermal *S. aureus* injection models. But in a physiological setting, keratinocytes are the first cells to encounter *S. aureus* and they need to orchestrate subsequent chemokine and cytokine production to recruit neutrophils to the site of infection (see Accepted Publication III, (Bitschar et al., 2017)). In fact, in a recent publication, Liu et al. compared epicutaneous to intradermal *S. aureus* infection and the results show that critical players involved in bacterial clearance such as MyD88 have adverse effects when *S. aureus* is applied epicutaneously (Liu et al., 2017).

In order to argue against objections, we decided to confirm the neutrophil-mediated elevation of *S. aureus* skin colonization by establishing an *in vitro* co-culture assay: In the co-culture model PHKs were cultivated in 0.4 µm cell culture inserts and primary polymorphonuclear cells (PMNs) or peripheral blood mononuclear cells (PBMCs) were added to the bottom compartment for 18 hours. In the following, *S. aureus* was added to PHKs and the number of viable colonizing bacteria 1.5 hours after infection was determined (Accepted Manuscript I, Figure 4a). In line with our *in vivo* data we found that the presence of PMNs, but not PBMCs, significantly enhanced *S. aureus* adhesion to keratinocytes (Accepted Manuscript I, Figure 4b). This effect seems to be dependent on soluble factors since direct contact of PHKs and PMNs is prevented by the 0.4 µm insert. However, the effect could neither be observed when using PMN supernatant instead of living PMNs (Accepted Manuscript I, Figure S3b). Interestingly, pre-incubation of PMNs with PHKs is crucial since this effect could only be achieved upon PMN pre-incubation with PHKs and presence during the infection. Elevated *S. aureus* levels were not observed without pre-incubation or sole presence of PMNs during the 1.5 hours of infection (Accepted Manuscript I, Figure 4b and Figure S3a). In summary, these data suggest that soluble factors secreted by *S. aureus*-infected keratinocytes induce PMNs to produce factors that create a favoring environment for *S. aureus*.

We further investigated whether the effect could also be achieved by the commensal *S. epidermidis*. However, neither in the *in vitro* co-culture assay nor in the *in vivo* model upon tape-stripping enhanced colonization of *S. epidermidis* was

observed (Accepted Manuscript I, Figure 4c+d) indicating that the neutrophil-mediated enhancement of skin colonization is specific for *S. aureus*. In an attempt to identify the *S. aureus*-specific factors involved in neutrophil-mediated enhancement of *S. aureus* skin colonization we used the USA300 Δ*agr*Δ*sae* double mutant (Munzenmayer et al., 2016), lacking virulence factors, and an USA300 Δ*lgt* mutant, lacking lipopeptides, in our co-culture model. However, as already indicated by enhanced colonization with the *agr*-deficient strain SA113 upon tape-stripping (Accepted Manuscript I, Figure 1a), neither of these factors contributed to enhanced *S. aureus* colonization *in vitro* (Accepted Manuscript I, Figure 4e). This was further confirmed *in vivo* by the usage of mice deficient for MyD88, TLR2, and TLR9 which did not show higher levels of *S. aureus* colonization than WT mice upon tape-stripping (Accepted Manuscript I, Figure 4f).

To summarize, our data clearly demonstrate that presence of neutrophils *in vivo* and *in vitro* enhances *S. aureus* skin colonization. By contrast, Malhotra et al. (2016) show that neutrophil-depleted mice display a significant defect in clearing epicutaneous *S. aureus* from tape-stripped skin. However, in this work *S. aureus* was applied 24 hours after tape-stripping and *S. aureus* colonization was analyzed 5 days after colonization (Malhotra et al., 2016). Taken together our data and the data from Malhotra et al. (2016) it is possible that the initial contribution of neutrophils to *S. aureus* colonization represents a local safeguard mechanism that tolerates enhanced colonization but at the same time prevents spreading of *S. aureus* in order to provide the time needed until a full adaptive immune response has developed. However, to confirm this hypothesis, time kinetics of immune cell compositions in the skin as well as of local and systemic *S. aureus* levels are needed.

7.3.3 Neutrophil extracellular traps are responsible for increased *S. aureus* skin colonization

In the following, we wanted to identify the neutrophilic factors mediating enhanced *S. aureus* colonization. In order to identify the responsible factors, we treated the PMN compartment in the co-culture model with DNAseI, protease inhibitors, phorbol-myristate-actetat (PMA), the calcium ionophore ionomycin or the formylated peptide N-formyl-met-leu-phe (fMLF) (Accepted Manuscript I, Figure S4). Interestingly, only DNAseI treatment prevented an increase in *S. aureus* colonization in the co-culture model, whereas the other compounds used did not alter *S. aureus* colonization of

PHKs (Accepted Manuscript I, Figure 5a and Figure S4a). Of note, in the absence of PMNs, treatment of only PHKs with the different compounds did not affect *S. aureus* colonization (Accepted Manuscript I, Figure S4a) suggesting that neutrophils release DNA that consequently drives enhanced *S. aureus* colonization.

DNA can be released by neutrophils in the form of NETs in response to microbial infection including *S. aureus*. These NETs are large, extracellular web-like structures which are released in order to trap and kill microbes and consist of decondensed chromatin covered with AMPs (Branzk et al., 2014; Brinkmann et al., 2004; Papayannopoulos and Zychlinsky, 2009) (see section 5.1.3). Since DNAseI treatment reverted enhanced *S. aureus* colonization, we hypothesized that NETs might be responsible for the observed increase in colonization. Thus, we analyzed the presence of NETs in the co-culture system by SYTOX™ staining as well as by an anti-double-stranded DNA (dsDNA) ELISA (Accepted Manuscript I, Figure 5b+c). In the SYTOX™ assay PMA treatment of PMNs was used as a positive control and resulted in a strong release of DNA which could be reverted by DNAseI treatment (Accepted Manuscript I, Figure 5b). Indeed, we could observe DNA release by PMNs in our co-culture setting upon *S. aureus* infection of PHKs (Accepted Manuscript I, Figure 5b). These data were confirmed by the anti-dsDNA ELISA where *S. aureus* infection of PHKs in the co-culture system also resulted in a strong release of dsDNA which could be reverted by the addition of DNAseI (Accepted Manuscript I, Figure 5c).

Since many microorganisms such as *S. aureus* have evolved different mechanisms that allow them to survive and escape neutrophil-mediated phagocytosis (Guerra et al., 2017) it can be assumed that NETs constitute an alternative tool used by neutrophils to kill these virulent microorganisms. Consistent with this idea, extracellular DNA measured by SYTOX™ was only observed when PHKs were infected with *S. aureus* and not upon infection with the skin commensal *S. epidermidis* (Accepted Manuscript I, Figure 5b). Neither did tape-stripping increase *S. epidermidis* loads on mouse skin (Accepted Manuscript I, Figure 1a). In line with this, also other groups have demonstrated that virulent entero-pathogenic bacteria induce NET formation whereas non-virulent probiotic bacteria do not (Vong et al., 2014). To further confirm this observation, we performed confocal laser scanning microscopy (LSM) of PMNs which were either infected with *S. aureus* or *S. epidermidis* or treated with PMA as a positive control. Staining for citrullinated histone

H3 (citH3) and MPO, two NET components (Neeli et al., 2008; Papayannopoulos et al., 2010), confirmed that *S. aureus* infection of neutrophils indeed results in NET formation whereas *S. epidermidis* is phagocytosed (Accepted Manuscript I, Figure 5d). Furthermore, to ultimately prove that the extracellular DNA observed by SYTOX™ and dsDNA ELISA in the co-culture model equals NETs, we additionally performed confocal LSM of PMNs in the co-culture setting. Indeed, we found NET-forming PMNs only upon *S. aureus* infection of PHKs and not upon infection with *S. epidermidis* (Accepted Manuscript I, Figure 5d). In order to provide final proof that NETs are responsible for increased *S. aureus* colonization of PHKs, we purified NETs from PMA-stimulated PMNs and co-cultured PHKs with these NETs instead of PMNs (Accepted Manuscript I, Figure 5e). Of note, purified NETs did not decrease PHK viability in the concentrations tested (Accepted Manuscript I, Figure 5f). Indeed, purified NETs increased *S. aureus* colonization of PHKs in a concentration-dependent manner. This increase was comparable to the increase achieved by PMNs and could also be reverted by DNAseI treatment (Accepted Manuscript I, Figure 5e). To ultimately confirm that neutrophil-mediated NET release increases *S. aureus* colonization of PHKs, we additionally used two inhibitors, GSK484 and Cl-Amidine, both targeting protein arginine deiminase 4 (PAD4), a critical player in NET formation (Papayannopoulos, 2018). These compounds effectively inhibited the release of extracellular DNA measured by SYTOX™ (Accepted Manuscript I, Figure S4b) and in the co-culture setting these inhibitors effectively prevented enhanced *S. aureus* colonization (Accepted Manuscript I, Figure 5g) further confirming that NETs cause the enhanced *S. aureus* colonization. Moreover, addition of neutrophilic genomic DNA (gDNA) to the co-culture nicely demonstrated that the effect is specific for NETs and cannot be exerted by other forms of DNA (Accepted Manuscript I, Figure 5g). In conclusion, these data show that *S. aureus* infection of PHKs induces the release of NETs from PMNs which consequently results in enhanced PHK colonization with *S. aureus.*

Our observation is supported by the findings of Yipp et al. (2012) who used spinning-disk confocal intravital microscopy to observe NET formation upon *S. aureus* skin infection. Disruption of these NETs by DNAseI treatment of mice, however, resulted in increased bacteremia but reduced *S. aureus* loads on the skin indicating that NETs may be important for containment and prevention of acute

bloodstream infections but that they seem counterproductive in skin infections (Yipp et al., 2012).

The work presented here illustrates that even though NETs may locally contain *S. aureus* infections, they also cause pathology by increasing *S. aureus* colonization. Of note, NETs have already been shown to directly damage epithelial and endothelial cells (Saffarzadeh et al., 2012; Villanueva et al., 2011) indicating that NET formation and clearance needs to be tightly regulated to prevent host cell damage. In our setting, however, NETs did not directly affect PHK viability (Accepted Manuscript I, Figure 5f). Considerably, NETs have also been associated with different pathologies such as *Pseudomonas aeruginosa* infections in cystic fibrosis patients (Yoo et al., 2014), systemic lupus erythematosus (Garcia-Romo et al., 2011) or rheumatoid arthritis (Khandpur et al., 2013). A role for NET formation in AD patients suffering from *S. aureus* infections, however, has not been established yet. Future studies should aim at elucidating the signaling pathways that drive NET formation in epicutaneous *S. aureus* infection as well as at identifying the pathways that NETs induce in PHKs in order to explain how *S. aureus* colonization is increased.

Of note, it was shown that tape-stripping itself without *S. aureus* infection already enhances NET formation of neutrophils in the skin which might additionally contribute to the enhanced *S. aureus* colonization observed (Guiducci et al., 2010). Notably, NET formation was also reported to be carried out, even though to a minor extend, by other cell types such as monocytes (Webster et al., 2010), macrophages (Okubo et al., 2018) or mast cells (Abel et al., 2011; Naqvi et al., 2017; von Kockritz-Blickwede et al., 2008). In our study we also found a significant increase of monocytes and slightly elevated levels of macrophages in mouse skin 24 hours upon tape-stripping indicating that NET formation by other cell types, which were not investigated in more detail in this study, might additionally contribute to enhanced *S. aureus* colonization observed *in vivo*.

Even though a role for *S. aureus* virulence factors was excluded in our experimental setting (Accepted Manuscript I, Figure 4e), virulence factors have been shown to contribute to NET formation and might thus play a role in a physiological setting: For example, low concentrations of PVL and LukGH have been shown to prime neutrophils and induce NET formation (Graves et al., 2012; Malachowa et al., 2013; Pilsczek et al., 2010). Additionally, bacterial-derived products such as N-formyl

peptides or the phenol-soluble modulins (PSMs) produced by *S. aureus* have the demonstrated ability to directly recruit neutrophils, followed by activation and lysis (Wang et al., 2007). Furthermore, *S. aureus*-mediated NET degradation with its staphylococcal nuclease and additional triggering of macrophage apoptosis with its virulence factor adenosine synthase A which convert NETs to 2'deoxyadenosine (Thammavongsa et al., 2013) might also contribute to *S. aureus* colonization. The factors that contribute to enhanced colonization in our model, however, need to be elucidated by future studies.

7.3.4 *S. epidermidis* prevents *S. aureus*-induced neutrophil recruitment

As it is shown in Accepted Publication I, pretreatment of mouse skin with the commensal *S. epidermidis* or with its secreted factors confers protection against *S. aureus* colonization. Based on the findings that neutrophils contribute to *S. aureus* colonization, we hypothesized that *S. epidermidis* might also modulate neutrophil recruitment during this protective process. Therefore, we used an *in vitro* Boyden Chamber assay to analyze the migrative capacity of PMNs in response to supernatant of PHKs treated with *S. aureus* only or pretreated with *S. epidermidis* CM followed by *S. aureus* treatment. Indeed, while supernatant of PHKs treated with *S. epidermidis* or its secreted factors induced a lower and non-significant recruitment of PMNs in the Boyden Chamber migration assay compared to *S. aureus*-infected PHKs, pretreatment of PHKs with *S. epidermidis* CM significantly reduced the number of migrating PMNs induced by *S. aureus* infection (Accepted Manuscript I, Figure 6a). To confirm these results *in vivo*, we epicutaneously pretreated mouse skin with *S. epidermidis* or PBS as a control followed by *S. aureus* infection 24 hours later. The next day, the number of neutrophils in the skin of these mice was analyzed. Indeed, *S. epidermidis* CM significantly prevented the recruitment of neutrophils to the skin in response to *S. aureus*, indicating that the prevention of neutrophil recruitment also contributes to *S. epidermidis*-mediated *S. aureus* protection *in vivo* (Accepted Manuscript I, Figure 6b). Conclusively, these results illustrate that *S. epidermidis* modulates the release of neutrophil-recruiting cytokines and chemokines in mouse skin. In fact, the reduced neutrophil recruitment also correlated with a reduction in pro-inflammatory cytokines induced by *S. aureus* such as TNFα, IL-6, GM-CSF, IL-17A or IL-12p70 and others in mouse skin (Accepted Manuscript I,

Figure 6c). Other cytokines such as MCP-1, IL-10 and MIP-2 were not significantly changed by *S. epidermidis* pretreatment (Accepted Manuscript I, Figure S5).

In summary, the results presented in this work might partially explain why *S. epidermidis* is able to protect the skin against *S. aureus* colonization (Accepted Publication I, (Burian et al., 2017)). However, whether *S. epidermidis* also interferes with NET formation capacities of neutrophils in mouse skin remains to be determined by future studies. Moreover, it remains elusive why *S. epidermidis* does not protect the skin during skin inflammation. Therefore, neutrophil recruitment in response to tape-stripping needs to be analyzed upon pretreatment with *S. epidermidis*. However, it was also shown that skin inflammation in the form of tape-stripping alters the composition of the microbiome (Karisola et al., 2019; Zeeuwen et al., 2012) which might be another factor to consider that might interfere with the commensal's ability to modulate neutrophil recruitment.

7.3.5 Conclusion

Even though NETs are extruded from neutrophils in order to trap and kill microbes, this work intriguingly demonstrates that enhanced presence of neutrophils in murine skin or in a human *in vitro* co-culture system of keratinocytes and neutrophils and concomitant NET formation enhances the ability of *S. aureus* to colonize the skin. *S. aureus* seems to benefit from NET formation as it might generate a new microbial niche in inflamed areas of the epithelial barrier, which might be exploited for colonization. Another possibility to explain this phenomenon is that it might represent a host-initiated safeguard mechanism by which allowance to temporarily colonize the skin might provide the additional time needed to initiate an adaptive immune response. During this time the infection may be kept locally under control and systemic infections may become rather unlikely. Finally, we present a model (Figure 9) in which an alliance of keratinocytes and the skin microbiota collectively prevents *S. aureus* from colonizing the skin during homeostasis. However, upon colonization with *S. aureus*, due to skin inflammation or dysbiosis, neutrophils are being recruited and undergo NET formation which ultimately enhances *S. aureus* levels on the skin. Future studies are necessary to resolve the mechanistic details in this scenario.

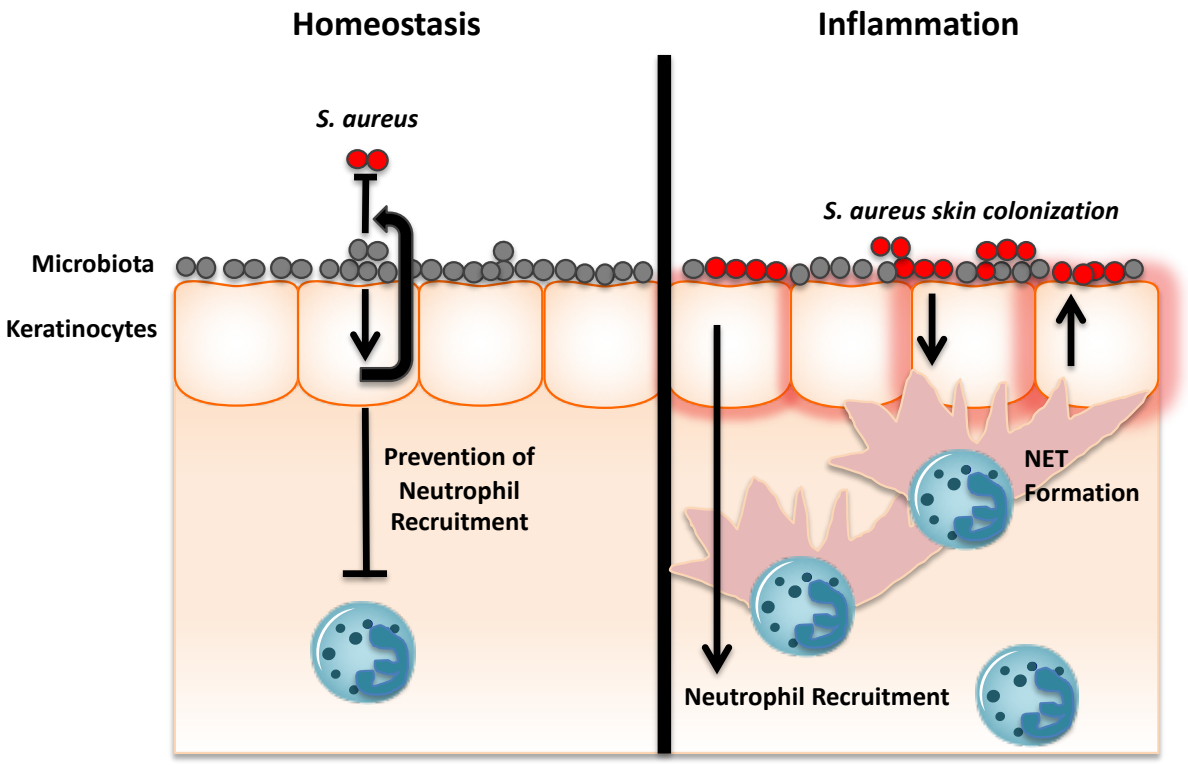


Figure 9: Proposed model of the role of neutrophils during epicutaneous *S. aureus* skin colonization (Accepted Manuscript I)

In healthy skin (left panel) concordant action of keratinocytes and the skin microbiota prevents *S. aureus* from colonizing the skin by reducing the recruitment of neutrophils. However, during skin inflammation or upon *S. aureus* colonization (right panel), neutrophils are recruited to the skin where they undergo NET formation resulting in enhanced *S. aureus* colonization of the skin.

7.4 Conclusive remarks and future perspectives

Bacterial interference by commensals that leads to inhibition of *S. aureus* skin colonization is a multifactorial phenomenon that we are only beginning to understand. It is likely that several independent mechanisms overlay each other adding to the resistance to *S. aureus* colonization. The mechanisms described here, executed by two members of skin commensals, *S. lugdunensis* and *S. epidermidis*, can only partly explain the mechanisms that prevent *S. aureus* from colonizing. There are myriads of other commensal microbes that were attributed skin-governing effects and there might be a multitude of other peptide antibiotics produced by our own commensals that also target *S. aureus* as well as other pathogens. Additionally, the mechanisms described in this work seem to be context-dependent: While *S. epidermidis* significantly protects healthy skin from *S. aureus*, *S. epidermidis* seems to even contribute further to *S. aureus* colonization during skin inflammation. Moreover, this work illustrates that also the outcome of immune cell recruitment is context-dependent and its consequences can be opposing: While lugdunin-induced neutrophil recruitment likely contributes to reduced *S. aureus* colonization, the presence of neutrophils upon tape-stripping as well as in our *in vitro* colonization model results in enhanced *S. aureus* colonization. For future studies it will be important to investigate how the ability of other skin commensals to produce antimicrobial molecules and modulate skin immunity contributes to *S. aureus* colonization resistance and whether the absence of such mechanisms may promote the development of AD.

8 References

Abel, J., Goldmann, O., Ziegler, C., Holtje, C., Smeltzer, M.S., Cheung, A.L., Bruhn, D., Rohde, M., and Medina, E. (2011). Staphylococcus aureus evades the extracellular antimicrobial activity of mast cells by promoting its own uptake. J Innate Immun *3*, 495-507.

Abrahamsson, T.R., Jakobsson, H.E., Andersson, A.F., Bjorksten, B., Engstrand, L., and Jenmalm, M.C. (2012). Low diversity of the gut microbiota in infants with atopic eczema. J Allergy Clin Immunol *129*, 434-440, 440 e431-432.

Adams, E.J., Gu, S., and Luoma, A.M. (2015). Human gamma delta T cells: Evolution and ligand recognition. Cell Immunol *296*, 31-40.

Afonina, I.S., Muller, C., Martin, S.J., and Beyaert, R. (2015). Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme. Immunity *42*, 991-1004.

Afonina, I.S., Tynan, G.A., Logue, S.E., Cullen, S.P., Bots, M., Luthi, A.U., Reeves, E.P., McElvaney, N.G., Medema, J.P., Lavelle, E.C.*, et al.* (2011). Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. Mol Cell *44*, 265-278.

Akira, S., Takeda, K., and Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol *2*, 675-680.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. Cell *124*, 783-801.

Akiyama, T., Niyonsaba, F., Kiatsurayanon, C., Nguyen, T.T., Ushio, H., Fujimura, T., Ueno, T., Okumura, K., Ogawa, H., and Ikeda, S. (2014). The human cathelicidin LL-37 host defense peptide upregulates tight junction-related proteins and increases human epidermal keratinocyte barrier function. J Innate Immun *6*, 739-753.

Albanesi, C., De Pita, O., and Girolomoni, G. (2007). Resident skin cells in psoriasis: a special look at the pathogenetic functions of keratinocytes. Clin Dermatol *25*, 581-588.

Ali, N., Zirak, B., Rodriguez, R.S., Pauli, M.L., Truong, H.A., Lai, K., Ahn, R., Corbin, K., Lowe, M.M., Scharschmidt, T.C.*, et al.* (2017). Regulatory T Cells in Skin Facilitate Epithelial Stem Cell Differentiation. Cell *169*, 1119-1129 e1111.

Amagai, M., Matsuyoshi, N., Wang, Z.H., Andl, C., and Stanley, J.R. (2000). Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. Nature medicine *6*, 1275-1277.

Archer, N.K., Jo, J.H., Lee, S.K., Kim, D., Smith, B., Ortines, R.V., Wang, Y., Marchitto, M.C., Ravipati, A., Cai, S.S.*, et al.* (2019). Injury, dysbiosis, and filaggrin deficiency drive skin inflammation through keratinocyte IL-1alpha release. J Allergy Clin Immunol *143*, 1426-1443 e1426.

Argemi, X., Hansmann, Y., Prola, K., and Prevost, G. (2019). Coagulase-Negative Staphylococci Pathogenomics. Int J Mol Sci *20*.

Artis, D., and Spits, H. (2015). The biology of innate lymphoid cells. Nature *517*, 293-301.

Asanuma, M., Kurokawa, K., Ichikawa, R., Ryu, K.H., Chae, J.H., Dohmae, N., Lee, B.L., and Nakayama, H. (2011). Structural evidence of alpha-aminoacylated lipoproteins of Staphylococcus aureus. FEBS J *278*, 716-728.

Askarian, F., van Sorge, N.M., Sangvik, M., Beasley, F.C., Henriksen, J.R., Sollid, J.U., van Strijp, J.A., Nizet, V., and Johannessen, M. (2014). A Staphylococcus aureus TIR domain protein virulence factor blocks TLR2-mediated NF-kappaB signaling. J Innate Immun *6*, 485-498.

Atmatzidis, D.H., Lambert, W.C., and Lambert, M.W. (2017). Langerhans cell: exciting developments in health and disease. J Eur Acad Dermatol Venereol *31*, 1817-1824.

Barton, G.M., Kagan, J.C., and Medzhitov, R. (2006). Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA. Nat Immunol *7*, 49-56.

Beck, L.A., Thaci, D., Hamilton, J.D., Graham, N.M., Bieber, T., Rocklin, R., Ming, J.E., Ren, H., Kao, R., Simpson, E.*, et al.* (2014). Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl J Med *371*, 130-139.

Becker, K., Heilmann, C., and Peters, G. (2014). Coagulase-negative staphylococci. Clin Microbiol Rev *27*, 870-926.

Becucci, L., Valensin, D., Innocenti, M., and Guidelli, R. (2014). Dermcidin, an anionic antimicrobial peptide: influence of lipid charge, pH and Zn2+ on its interaction with a biomimetic membrane. Soft Matter *10*, 616-626.

Bengtsson, T., Lonn, J., Khalaf, H., and Palm, E. (2018). The lantibiotic gallidermin acts bactericidal against Staphylococcus epidermidis and Staphylococcus aureus and antagonizes the bacteria-induced proinflammatory responses in dermal fibroblasts. Microbiologyopen.

Bieber, L., and Kahlmeter, G. (2010). Staphylococcus lugdunensis in several niches of the normal skin flora. Clin Microbiol Infect *16*, 385-388.

Bieber, T. (2008). Atopic dermatitis. N Engl J Med *358*, 1483-1494.

Bin, L., and Leung, D.Y. (2016). Genetic and epigenetic studies of atopic dermatitis. Allergy Asthma Clin Immunol *12*, 52.

Bitschar, K., Sauer, B., Focken, J., Dehmer, H., Moos, S., Konnerth, M., Schilling, N.A., Grond, S., Kalbacher, H., Kurschus, F.C.*, et al.* (2019). Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. Nat Commun *10*, 2730.

Bitschar, K., Wolz, C., Krismer, B., Peschel, A., and Schittek, B. (2017). Keratinocytes as sensors and central players in the immune defense against Staphylococcus aureus in the skin. J Dermatol Sci *87*, 215-220.

Bjerketorp, J., Nilsson, M., Ljungh, A., Flock, J.I., Jacobsson, K., and Frykberg, L. (2002). A novel von Willebrand factor binding protein expressed by Staphylococcus aureus. Microbiology *148*, 2037-2044.

Bloes, D.A., Kretschmer, D., and Peschel, A. (2015). Enemy attraction: bacterial agonists for leukocyte chemotaxis receptors. Nature reviews Microbiology *13*, 95-104.

Bode, L.G., Kluytmans, J.A., Wertheim, H.F., Bogaers, D., Vandenbroucke-Grauls, C.M., Roosendaal, R., Troelstra, A., Box, A.T., Voss, A., van der Tweel, I.*, et al.* (2010). Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med *362*, 9-17.

Boeltz, S., Amini, P., Anders, H.J., Andrade, F., Bilyy, R., Chatfield, S., Cichon, I., Clancy, D.M., Desai, J., Dumych, T.*, et al.* (2019). To NET or not to NET:current opinions and state of the science regarding the formation of neutrophil extracellular traps. Cell Death Differ *26*, 395-408.

Branzk, N., Lubojemska, A., Hardison, S.E., Wang, Q., Gutierrez, M.G., Brown, G.D., and Papayannopoulos, V. (2014). Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol *15*, 1017-1025.

Brauweiler, A.M., Goleva, E., and Leung, D.Y.M. (2019). Staphylococcus aureus Lipoteichoic Acid Damages the Skin Barrier through an IL-1 mediated Pathway. J Invest Dermatol.

Breukink, E., van Heusden, H.E., Vollmerhaus, P.J., Swiezewska, E., Brunner, L., Walker, S., Heck, A.J., and de Kruijff, B. (2003). Lipid II is an intrinsic component of the pore induced by nisin in bacterial membranes. J Biol Chem *278*, 19898-19903.

Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., and Zychlinsky, A. (2004). Neutrophil extracellular traps kill bacteria. Science *303*, 1532-1535.

Britigan, B.E., Hassett, D.J., Rosen, G.M., Hamill, D.R., and Cohen, M.S. (1989). Neutrophil degranulation inhibits potential hydroxyl-radical formation. Relative impact of myeloperoxidase and lactoferrin release on hydroxyl-radical production by iron-supplemented neutrophils assessed by spin-trapping techniques. Biochem J *264*, 447-455.

Brotz, H., Josten, M., Wiedemann, I., Schneider, U., Gotz, F., Bierbaum, G., and Sahl, H.G. (1998). Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol Microbiol *30*, 317-327.

Brown, S.J. (2017). Molecular mechanisms in atopic eczema: insights gained from genetic studies. J Pathol *241*, 140-145.

Brown, T.M., and Krishnamurthy, K. (2019). Histology, Dermis. In StatPearls (Treasure Island (FL)).

Buffie, C.G., and Pamer, E.G. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol *13*, 790-801.

Bunikowski, R., Mielke, M., Skarabis, H., Herz, U., Bergmann, R.L., Wahn, U., and Renz, H. (1999). Prevalence and role of serum IgE antibodies to the Staphylococcus aureus-derived superantigens SEA and SEB in children with atopic dermatitis. J Allergy Clin Immunol *103*, 119-124.

Burian, M., Bitschar, K., Dylus, B., Peschel, A., and Schittek, B. (2017). The Protective Effect of Microbiota on S. aureus Skin Colonization Depends on the Integrity of the Epithelial Barrier. J Invest Dermatol *137*, 976-979.

Byrd, A.L., Belkaid, Y., and Segre, J.A. (2018). The human skin microbiome. Nat Rev Microbiol *16*, 143-155.

Byrd, A.L., Deming, C., Cassidy, S.K.B., Harrison, O.J., Ng, W.I., Conlan, S., Program, N.C.S., Belkaid, Y., Segre, J.A., and Kong, H.H. (2017). Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis. Sci Transl Med *9*.

Candi, E., Schmidt, R., and Melino, G. (2005). The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol *6*, 328-340.

Carretero, M., Escamez, M.J., Garcia, M., Duarte, B., Holguin, A., Retamosa, L., Jorcano, J.L., Rio, M.D., and Larcher, F. (2008). In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37. J Invest Dermatol *128*, 223-236.

Chan, L.S., Robinson, N., and Xu, L. (2001). Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol *117*, 977-983.

Chi, C.Y., Lin, C.C., Liao, I.C., Yao, Y.C., Shen, F.C., Liu, C.C., and Lin, C.F. (2014). Panton-Valentine leukocidin facilitates the escape of Staphylococcus aureus from human keratinocyte endosomes and induces apoptosis. The Journal of infectious diseases *209*, 224-235.

Chiang, N., Fredman, G., Backhed, F., Oh, S.F., Vickery, T., Schmidt, B.A., and Serhan, C.N. (2012). Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature *484*, 524-528.

Cho, J.S., Pietras, E.M., Garcia, N.C., Ramos, R.I., Farzam, D.M., Monroe, H.R., Magorien, J.E., Blauvelt, A., Kolls, J.K., Cheung, A.L.*, et al.* (2010). IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J Clin Invest *120*, 1762-1773.

Chorro, L., Sarde, A., Li, M., Woollard, K.J., Chambon, P., Malissen, B., Kissenpfennig, A., Barbaroux, J.B., Groves, R., and Geissmann, F. (2009). Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and

inflammation-associated expansion of the epidermal LC network. J Exp Med *206*, 3089-3100.

Choy, D.F., Hsu, D.K., Seshasayee, D., Fung, M.A., Modrusan, Z., Martin, F., Liu, F.T., and Arron, J.R. (2012). Comparative transcriptomic analyses of atopic dermatitis and psoriasis reveal shared neutrophilic inflammation. J Allergy Clin Immunol *130*, 1335-1343 e1335.

Clark, R.A., Chong, B., Mirchandani, N., Brinster, N.K., Yamanaka, K., Dowgiert, R.K., and Kupper, T.S. (2006). The vast majority of CLA+ T cells are resident in normal skin. J Immunol *176*, 4431-4439.

Clarke, T.B., Davis, K.M., Lysenko, E.S., Zhou, A.Y., Yu, Y., and Weiser, J.N. (2010). Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat Med *16*, 228-231.

Clausen, M.L., Edslev, S.M., Norreslet, L.B., Sorensen, J.A., Andersen, P.S., and Agner, T. (2019). Temporal variation of Staphylococcus aureus clonal complexes in atopic dermatitis: a follow-up study. Br J Dermatol *180*, 181-186.

Cogen, A.L., Yamasaki, K., Sanchez, K.M., Dorschner, R.A., Lai, Y., MacLeod, D.T., Torpey, J.W., Otto, M., Nizet, V., Kim, J.E.*, et al.* (2010). 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.

Cohen, T.S., Jones-Nelson, O., Hotz, M., Cheng, L., Miller, L.S., Suzich, J., Stover, C.K., and Sellman, B.R. (2016). S. aureus blocks efferocytosis of neutrophils by macrophages through the activity of its virulence factor alpha toxin. Sci Rep *6*, 35466.

Conlan, S., Mijares, L.A., Program, N.C.S., Becker, J., Blakesley, R.W., Bouffard, G.G., Brooks, S., Coleman, H., Gupta, J., Gurson, N.*, et al.* (2012). Staphylococcus epidermidis pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates. Genome Biol *13*, R64.

Cornelissen, C., Marquardt, Y., Czaja, K., Wenzel, J., Frank, J., Luscher-Firzlaff, J., Luscher, B., and Baron, J.M. (2012). IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. J Allergy Clin Immunol *129*, 426-433, 433 e421-428.

Costello, E.K., Lauber, C.L., Hamady, M., Fierer, N., Gordon, J.I., and Knight, R. (2009). Bacterial community variation in human body habitats across space and time. Science *326*, 1694-1697.

Czarnowicki, T., Esaki, H., Gonzalez, J., Malajian, D., Shemer, A., Noda, S., Talasila, S., Berry, A., Gray, J., Becker, L.*, et al.* (2015). Early pediatric atopic dermatitis shows only a cutaneous lymphocyte antigen (CLA)(+) TH2/TH1 cell imbalance, whereas adults acquire CLA(+) TH22/TC22 cell subsets. J Allergy Clin Immunol *136*, 941-951 e943.

d'Ersu, J., Aubin, G.G., Mercier, P., Nicollet, P., Bemer, P., and Corvec, S. (2016). Characterization of Staphylococcus caprae Clinical Isolates Involved in Human Bone

and Joint Infections, Compared with Goat Mastitis Isolates. J Clin Microbiol *54*, 106-113.

Daillere, R., Vetizou, M., Waldschmitt, N., Yamazaki, T., Isnard, C., Poirier-Colame, V., Duong, C.P.M., Flament, C., Lepage, P., Roberti, M.P.*, et al.* (2016). Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects. Immunity *45*, 931-943.

Dambuza, I.M., and Brown, G.D. (2015). C-type lectins in immunity: recent developments. Curr Opin Immunol *32*, 21-27.

de Haas, C.J., Veldkamp, K.E., Peschel, A., Weerkamp, F., Van Wamel, W.J., Heezius, E.C., Poppelier, M.J., Van Kessel, K.P., and van Strijp, J.A. (2004). Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Exp Med *199*, 687-695.

de Oliveira, S., Rosowski, E.E., and Huttenlocher, A. (2016). Neutrophil migration in infection and wound repair: going forward in reverse. Nat Rev Immunol *16*, 378-391.

Dean, S.N., Bishop, B.M., and van Hoek, M.L. (2011). Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against Staphylococcus aureus. BMC Microbiol *11*, 114.

Deckers, I.A., McLean, S., Linssen, S., Mommers, M., van Schayck, C.P., and Sheikh, A. (2012). Investigating international time trends in the incidence and prevalence of atopic eczema 1990-2010: a systematic review of epidemiological studies. PLoS One *7*, e39803.

DeLeo, F.R., Otto, M., Kreiswirth, B.N., and Chambers, H.F. (2010). Community-associated meticillin-resistant Staphylococcus aureus. Lancet *375*, 1557-1568.

Dhingra, N., Suarez-Farinas, M., Fuentes-Duculan, J., Gittler, J.K., Shemer, A., Raz, A., Fischetti, V.A., Krueger, J.G., and Guttman-Yassky, E. (2013). Attenuated neutrophil axis in atopic dermatitis compared to psoriasis reflects TH17 pathway differences between these diseases. J Allergy Clin Immunol *132*, 498-501 e493.

Di Meglio, P., Duarte, J.H., Ahlfors, H., Owens, N.D., Li, Y., Villanova, F., Tosi, I., Hirota, K., Nestle, F.O., Mrowietz, U.*, et al.* (2014). Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. Immunity *40*, 989-1001.

Dickel, H., Gambichler, T., Kamphowe, J., Altmeyer, P., and Skrygan, M. (2010). Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. Contact Dermatitis *63*, 215-222.

Diekema, D.J., Richter, S.S., Heilmann, K.P., Dohrn, C.L., Riahi, F., Tendolkar, S., McDanel, J.S., and Doern, G.V. (2014). Continued emergence of USA300 methicillin-resistant Staphylococcus aureus in the United States: results from a nationwide surveillance study. Infect Control Hosp Epidemiol *35*, 285-292.

Dischinger, J., Basi Chipalu, S., and Bierbaum, G. (2014). Lantibiotics: promising candidates for future applications in health care. Int J Med Microbiol *304*, 51-62.

Dobson, A., Cotter, P.D., Ross, R.P., and Hill, C. (2012). Bacteriocin production: a probiotic trait? Appl Environ Microbiol *78*, 1-6.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A *107*, 11971-11975.

Dong, Y., Speer, C.P., and Glaser, K. (2018). Beyond sepsis: Staphylococcus epidermidis is an underestimated but significant contributor to neonatal morbidity. Virulence *9*, 621-633.

Duan, J., Chung, H., Troy, E., and Kasper, D.L. (2010). Microbial colonization drives expansion of IL-1 receptor 1-expressing and IL-17-producing gamma/delta T cells. Cell Host Microbe *7*, 140-150.

Dudeck, A., Koberle, M., Goldmann, O., Meyer, N., Dudeck, J., Lemmens, S., Rohde, M., Roldan, N.G., Dietze-Schwonberg, K., Orinska, Z.*, et al.* (2018). Mast cells as protectors of health. J Allergy Clin Immunol.

Ebner, P., Reichert, S., Luqman, A., Krismer, B., Popella, P., and Gotz, F. (2018). Lantibiotic production is a burden for the producing staphylococci. Sci Rep *8*, 7471.

Edele, F., Molenaar, R., Gutle, D., Dudda, J.C., Jakob, T., Homey, B., Mebius, R., Hornef, M., and Martin, S.F. (2008). Cutting edge: instructive role of peripheral tissue cells in the imprinting of T cell homing receptor patterns. J Immunol *181*, 3745-3749.

Eyerich, K., Eyerich, S., and Biedermann, T. (2015). The Multi-Modal Immune Pathogenesis of Atopic Eczema. Trends Immunol *36*, 788-801.

Eyerich, S., Onken, A.T., Weidinger, S., Franke, A., Nasorri, F., Pennino, D., Grosber, M., Pfab, F., Schmidt-Weber, C.B., Mempel, M.*, et al.* (2011). Mutual antagonism of T cells causing psoriasis and atopic eczema. N Engl J Med *365*, 231-238.

Faith, J.J., Guruge, J.L., Charbonneau, M., Subramanian, S., Seedorf, H., Goodman, A.L., Clemente, J.C., Knight, R., Heath, A.C., Leibel, R.L.*, et al.* (2013). The long-term stability of the human gut microbiota. Science *341*, 1237439.

Figdor, C.G., van Kooyk, Y., and Adema, G.J. (2002). C-type lectin receptors on dendritic cells and Langerhans cells. Nat Rev Immunol *2*, 77-84.

Findley, K., Oh, J., Yang, J., Conlan, S., Deming, C., Meyer, J.A., Schoenfeld, D., Nomicos, E., Park, M., Program, N.I.H.I.S.C.C.S.*, et al.* (2013). Topographic diversity of fungal and bacterial communities in human skin. Nature *498*, 367-370.

Foolad, N., Brezinski, E.A., Chase, E.P., and Armstrong, A.W. (2013). Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. JAMA Dermatol *149*, 350-355.

Foster, T.J., Geoghegan, J.A., Ganesh, V.K., and Hook, M. (2014). Adhesion, invasion and evasion: the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol *12*, 49-62.

Foster, T.J., and Hook, M. (1998). Surface protein adhesins of Staphylococcus aureus. Trends Microbiol *6*, 484-488.

Fournier, B. (2012). The function of TLR2 during staphylococcal diseases. Front Cell Infect Microbiol *2*, 167.

Franchi, L., Eigenbrod, T., Munoz-Planillo, R., and Nunez, G. (2009). The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat Immunol *10*, 241-247.

Freeman, A.F., and Holland, S.M. (2010). Clinical manifestations of hyper IgE syndromes. Dis Markers *29*, 123-130.

Fuchs, E., and Raghavan, S. (2002). Getting under the skin of epidermal morphogenesis. Nat Rev Genet *3*, 199-209.

Fuchs, T.A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V., and Zychlinsky, A. (2007). Novel cell death program leads to neutrophil extracellular traps. J Cell Biol *176*, 231-241.

Fuentes-Duculan, J., Suarez-Farinas, M., Zaba, L.C., Nograles, K.E., Pierson, K.C., Mitsui, H., Pensabene, C.A., Kzhyshkowska, J., Krueger, J.G., and Lowes, M.A. (2010). A subpopulation of CD163-positive macrophages is classically activated in psoriasis. J Invest Dermatol *130*, 2412-2422.

Furue, M., and Kadono, T. (2019). The contribution of IL-17 to the development of autoimmunity in psoriasis. Innate Immun *25*, 337-343.

Furue, M., Yamamura, K., Kido-Nakahara, M., Nakahara, T., and Fukui, Y. (2018). Emerging role of interleukin-31 and interleukin-31 receptor in pruritus in atopic dermatitis. Allergy *73*, 29-36.

Fyhrquist, N., Ruokolainen, L., Suomalainen, A., Lehtimaki, S., Veckman, V., Vendelin, J., Karisola, P., Lehto, M., Savinko, T., Jarva, H.*, et al.* (2014). Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation. J Allergy Clin Immunol *134*, 1301-1309 e1311.

Gaffen, S.L. (2011). Recent advances in the IL-17 cytokine family. Curr Opin Immunol *23*, 613-619.

Ganguly, D., Chamilos, G., Lande, R., Gregorio, J., Meller, S., Facchinetti, V., Homey, B., Barrat, F.J., Zal, T., and Gilliet, M. (2009). Self-RNA-antimicrobial peptide complexes activate human dendritic cells through TLR7 and TLR8. J Exp Med *206*, 1983-1994.

Garcia-Romo, G.S., Caielli, S., Vega, B., Connolly, J., Allantaz, F., Xu, Z., Punaro, M., Baisch, J., Guiducci, C., Coffman, R.L.*, et al.* (2011). Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med *3*, 73ra20.

Gay, N.J., Symmons, M.F., Gangloff, M., and Bryant, C.E. (2014). Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol *14*, 546-558.

Geiger, T., Francois, P., Liebeke, M., Fraunholz, M., Goerke, C., Krismer, B., Schrenzel, J., Lalk, M., and Wolz, C. (2012). The stringent response of Staphylococcus aureus and its impact on survival after phagocytosis through the induction of intracellular PSMs expression. PLoS Pathog *8*, e1003016.

Ghigo, C., Mondor, I., Jorquera, A., Nowak, J., Wienert, S., Zahner, S.P., Clausen, B.E., Luche, H., Malissen, B., Klauschen, F.*, et al.* (2013). Multicolor fate mapping of Langerhans cell homeostasis. J Exp Med *210*, 1657-1664.

Gittler, J.K., Shemer, A., Suarez-Farinas, M., Fuentes-Duculan, J., Gulewicz, K.J., Wang, C.Q., Mitsui, H., Cardinale, I., de Guzman Strong, C., Krueger, J.G.*, et al.* (2012). Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J Allergy Clin Immunol *130*, 1344-1354.

Gong, J.Q., Lin, L., Lin, T., Hao, F., Zeng, F.Q., Bi, Z.G., Yi, D., and Zhao, B. (2006). Skin colonization by Staphylococcus aureus in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. Br J Dermatol *155*, 680-687.

Goris, H., de Boer, F., and van der Waaij, D. (1985). Myelopoiesis in experimentally contaminated specific-pathogen-free and germfree mice during oral administration of polymyxin. Infect Immun *50*, 437-441.

Graves, S.F., Kobayashi, S.D., Braughton, K.R., Whitney, A.R., Sturdevant, D.E., Rasmussen, D.L., Kirpotina, L.N., Quinn, M.T., and DeLeo, F.R. (2012). Sublytic concentrations of Staphylococcus aureus Panton-Valentine leukocidin alter human PMN gene expression and enhance bactericidal capacity. J Leukoc Biol *92*, 361-374.

Gray, E.E., Suzuki, K., and Cyster, J.G. (2011). Cutting edge: Identification of a motile IL-17-producing gammadelta T cell population in the dermis. J Immunol *186*, 6091-6095.

Greenlee-Wacker, M.C. (2016). Clearance of apoptotic neutrophils and resolution of inflammation. Immunol Rev *273*, 357-370.

Greenlee-Wacker, M.C., Rigby, K.M., Kobayashi, S.D., Porter, A.R., DeLeo, F.R., and Nauseef, W.M. (2014). Phagocytosis of Staphylococcus aureus by human neutrophils prevents macrophage efferocytosis and induces programmed necrosis. J Immunol *192*, 4709-4717.

Grice, E.A., Kong, H.H., Conlan, S., Deming, C.B., Davis, J., Young, A.C., Program, N.C.S., Bouffard, G.G., Blakesley, R.W., Murray, P.R.*, et al.* (2009). Topographical and temporal diversity of the human skin microbiome. Science *324*, 1190-1192.

Grice, E.A., Kong, H.H., Renaud, G., Young, A.C., Program, N.C.S., Bouffard, G.G., Blakesley, R.W., Wolfsberg, T.G., Turner, M.L., and Segre, J.A. (2008). A diversity profile of the human skin microbiota. Genome Res *18*, 1043-1050.

Grice, E.A., and Segre, J.A. (2011). The skin microbiome. Nat Rev Microbiol *9*, 244-253.

Guerra, F.E., Borgogna, T.R., Patel, D.M., Sward, E.W., and Voyich, J.M. (2017). Epic Immune Battles of History: Neutrophils vs. Staphylococcus aureus. Front Cell Infect Microbiol *7*, 286.

Guiducci, C., Tripodo, C., Gong, M., Sangaletti, S., Colombo, M.P., Coffman, R.L., and Barrat, F.J. (2010). Autoimmune skin inflammation is dependent on plasmacytoid dendritic cell activation by nucleic acids via TLR7 and TLR9. J Exp Med *207*, 2931-2942.

Han, H., Roan, F., and Ziegler, S.F. (2017). The atopic march: current insights into skin barrier dysfunction and epithelial cell-derived cytokines. Immunol Rev *278*, 116-130.

Hanel, K.H., Pfaff, C.M., Cornelissen, C., Amann, P.M., Marquardt, Y., Czaja, K., Kim, A., Luscher, B., and Baron, J.M. (2016). Control of the Physical and Antimicrobial Skin Barrier by an IL-31-IL-1 Signaling Network. J Immunol *196*, 3233-3244.

Hanifin, J.M., Reed, M.L., Eczema, P., and Impact Working, G. (2007). A population-based survey of eczema prevalence in the United States. Dermatitis *18*, 82-91.

Hannigan, G.D., Meisel, J.S., Tyldsley, A.S., Zheng, Q., Hodkinson, B.P., SanMiguel, A.J., Minot, S., Bushman, F.D., and Grice, E.A. (2015). The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. MBio *6*, e01578-01515.

Hanzelmann, D., Joo, H.S., Franz-Wachtel, M., Hertlein, T., Stevanovic, S., Macek, B., Wolz, C., Gotz, F., Otto, M., Kretschmer, D*., et al.* (2016). Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants. Nat Commun *7*, 12304.

Harder, J., Dressel, S., Wittersheim, M., Cordes, J., Meyer-Hoffert, U., Mrowietz, U., Folster-Holst, R., Proksch, E., Schroder, J.M., Schwarz, T*., et al.* (2010). Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. J Invest Dermatol *130*, 1355-1364.

Harkins, C.P., McAleer, M.A., Bennett, D., McHugh, M., Fleury, O.M., Pettigrew, K.A., Oravcova, K., Parkhill, J., Proby, C.M., Dawe, R.S*., et al.* (2018a). The widespread use of topical antimicrobials enriches for resistance in Staphylococcus aureus isolated from patients with atopic dermatitis. Br J Dermatol *179*, 951-958.

Harkins, C.P., Pettigrew, K.A., Oravcova, K., Gardner, J., Hearn, R.M.R., Rice, D., Mather, A.E., Parkhill, J., Brown, S.J., Proby, C.M*., et al.* (2018b). The Microevolution and Epidemiology of Staphylococcus aureus Colonization during Atopic Eczema Disease Flare. J Invest Dermatol *138*, 336-343.

Harrop, J., Chinn, S., Verlato, G., Olivieri, M., Norback, D., Wjst, M., Janson, C., Zock, J.P., Leynaert, B., Gislason, D*., et al.* (2007). Eczema, atopy and allergen exposure in adults: a population-based study. Clin Exp Allergy *37*, 526-535.

Hartman, B.J., and Tomasz, A. (1984). Low-affinity penicillin-binding protein associated with beta-lactam resistance in Staphylococcus aureus. J Bacteriol *158*, 513-516.

Hashimoto, M., Tawaratsumida, K., Kariya, H., Kiyohara, A., Suda, Y., Krikae, F., Kirikae, T., and Gotz, F. (2006). Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in Staphylococcus aureus. J Immunol *177*, 3162-3169.

Hasper, H.E., de Kruijff, B., and Breukink, E. (2004). Assembly and stability of nisin-lipid II pores. Biochemistry *43*, 11567-11575.

Hata, T.R., Kotol, P., Boguniewicz, M., Taylor, P., Paik, A., Jackson, M., Nguyen, M., Kabigting, F., Miller, J., Gerber, M.*, et al.* (2010). History of eczema herpeticum is associated with the inability to induce human beta-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. Br J Dermatol *163*, 659-661.

Hatano, Y., Adachi, Y., Elias, P.M., Crumrine, D., Sakai, T., Kurahashi, R., Katagiri, K., and Fujiwara, S. (2013). The Th2 cytokine, interleukin-4, abrogates the cohesion of normal stratum corneum in mice: implications for pathogenesis of atopic dermatitis. Exp Dermatol *22*, 30-35.

Hauser, C., Saurat, J.H., Schmitt, A., Jaunin, F., and Dayer, J.M. (1986). Interleukin 1 is present in normal human epidermis. J Immunol *136*, 3317-3323.

Henry, C.M., Sullivan, G.P., Clancy, D.M., Afonina, I.S., Kulms, D., and Martin, S.J. (2016). Neutrophil-Derived Proteases Escalate Inflammation through Activation of IL-36 Family Cytokines. Cell Rep *14*, 708-722.

Hirahara, K., Liu, L., Clark, R.A., Yamanaka, K., Fuhlbrigge, R.C., and Kupper, T.S. (2006). The majority of human peripheral blood CD4+CD25highFoxp3+ regulatory T cells bear functional skin-homing receptors. J Immunol *177*, 4488-4494.

Hirasawa, Y., Takai, T., Nakamura, T., Mitsuishi, K., Gunawan, H., Suto, H., Ogawa, T., Wang, X.L., Ikeda, S., Okumura, K.*, et al.* (2010). Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction. The Journal of investigative dermatology *130*, 614-617.

Hoebe, K., Georgel, P., Rutschmann, S., Du, X., Mudd, S., Crozat, K., Sovath, S., Shamel, L., Hartung, T., Zahringer, U.*, et al.* (2005). CD36 is a sensor of diacylglycerides. Nature *433*, 523-527.

Howell, M.D., Kim, B.E., Gao, P., Grant, A.V., Boguniewicz, M., DeBenedetto, A., Schneider, L., Beck, L.A., Barnes, K.C., and Leung, D.Y. (2009). Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol *124*, R7-R12.

Human Microbiome Project, C. (2012). Structure, function and diversity of the healthy human microbiome. Nature *486*, 207-214.

Hunter, C.A., and Jones, S.A. (2015). IL-6 as a keystone cytokine in health and disease. Nat Immunol *16*, 448-457.

Ide, F., Matsubara, T., Kaneko, M., Ichiyama, T., Mukouyama, T., and Furukawa, S. (2004). Staphylococcal enterotoxin-specific IgE antibodies in atopic dermatitis. Pediatr Int *46*, 337-341.

Igyarto, B.Z., Haley, K., Ortner, D., Bobr, A., Gerami-Nejad, M., Edelson, B.T., Zurawski, S.M., Malissen, B., Zurawski, G., Berman, J.*, et al.* (2011). Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. Immunity *35*, 260-272.

Igyarto, B.Z., and Kaplan, D.H. (2013). Antigen presentation by Langerhans cells. Curr Opin Immunol *25*, 115-119.

Iida, N., Dzutsev, A., Stewart, C.A., Smith, L., Bouladoux, N., Weingarten, R.A., Molina, D.A., Salcedo, R., Back, T., Cramer, S.*, et al.* (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science *342*, 967-970.

Irvine, A.D., McLean, W.H., and Leung, D.Y. (2011). Filaggrin mutations associated with skin and allergic diseases. N Engl J Med *365*, 1315-1327.

Ishii, K.J., Coban, C., Kato, H., Takahashi, K., Torii, Y., Takeshita, F., Ludwig, H., Sutter, G., Suzuki, K., Hemmi, H.*, et al.* (2006). A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat Immunol *7*, 40-48.

Ismail, I.H., Oppedisano, F., Joseph, S.J., Boyle, R.J., Licciardi, P.V., Robins-Browne, R.M., and Tang, M.L. (2012). Reduced gut microbial diversity in early life is associated with later development of eczema but not atopy in high-risk infants. Pediatr Allergy Immunol *23*, 674-681.

Ivanov, II, Atarashi, K., Manel, N., Brodie, E.L., Shima, T., Karaoz, U., Wei, D., Goldfarb, K.C., Santee, C.A., Lynch, S.V.*, et al.* (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell *139*, 485-498.

Ivanov, II, Frutos Rde, L., Manel, N., Yoshinaga, K., Rifkin, D.B., Sartor, R.B., Finlay, B.B., and Littman, D.R. (2008). Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe *4*, 337-349.

Iwamoto, K., Moriwaki, M., Miyake, R., and Hide, M. (2019). Staphylococcus aureus in atopic dermatitis: Strain-specific cell wall proteins and skin immunity. Allergol Int.

Iwamoto, K., Moriwaki, M., Niitsu, Y., Saino, M., Takahagi, S., Hisatsune, J., Sugai, M., and Hide, M. (2017). Staphylococcus aureus from atopic dermatitis skin alters cytokine production triggered by monocyte-derived Langerhans cell. J Dermatol Sci *88*, 271-279.

Iwase, T., Uehara, Y., Shinji, H., Tajima, A., Seo, H., Takada, K., Agata, T., and Mizunoe, Y. (2010). Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. Nature *465*, 346-349.

Iyoda, M., Shibata, T., Kawaguchi, M., Hizawa, N., Yamaoka, T., Kokubu, F., and Akizawa, T. (2010). IL-17A and IL-17F stimulate chemokines via MAPK pathways

(ERK1/2 and p38 but not JNK) in mouse cultured mesangial cells: synergy with TNF-alpha and IL-1beta. Am J Physiol Renal Physiol *298*, F779-787.

Janeway, C.A., Jr., and Medzhitov, R. (2002). Innate immune recognition. Annu Rev Immunol *20*, 197-216.

Jansen, K.U., Girgenti, D.Q., Scully, I.L., and Anderson, A.S. (2013). Vaccine review: "Staphyloccocus aureus vaccines: problems and prospects". Vaccine *31*, 2723-2730.

Jiang, X., Campbell, J.J., and Kupper, T.S. (2010). Embryonic trafficking of gammadelta T cells to skin is dependent on E/P selectin ligands and CCR4. Proc Natl Acad Sci U S A *107*, 7443-7448.

Jin, H., Oyoshi, M.K., Le, Y., Bianchi, T., Koduru, S., Mathias, C.B., Kumar, L., Le Bras, S., Young, D., Collins, M.*, et al.* (2009). IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. J Clin Invest *119*, 47-60.

Jin, M.S., Kim, S.E., Heo, J.Y., Lee, M.E., Kim, H.M., Paik, S.G., Lee, H., and Lee, J.O. (2007). Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell *130*, 1071-1082.

Kalali, B.N., Kollisch, G., Mages, J., Muller, T., Bauer, S., Wagner, H., Ring, J., Lang, R., Mempel, M., and Ollert, M. (2008). Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signaling. J Immunol *181*, 2694-2704.

Kang, J.Y., Nan, X., Jin, M.S., Youn, S.J., Ryu, Y.H., Mah, S., Han, S.H., Lee, H., Paik, S.G., and Lee, J.O. (2009). Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity *31*, 873-884.

Kaplan, D.H., Li, M.O., Jenison, M.C., Shlomchik, W.D., Flavell, R.A., and Shlomchik, M.J. (2007). Autocrine/paracrine TGFbeta1 is required for the development of epidermal Langerhans cells. J Exp Med *204*, 2545-2552.

Karisola, P., Suomalainen, A., Fortino, V., Ottman, N., Vendelin, J., Wolff, H.J., Ruokolainen, L., Greco, D., Fyhrquist, N., and Alenius, H. (2019). Tape-stripping alters the microbe-host correlations in mouse skin. Allergy *74*, 617-621.

Kato, A., Fujii, E., Watanabe, T., Takashima, Y., Matsushita, H., Furuhashi, T., and Morita, A. (2014). Distribution of IL-31 and its receptor expressing cells in skin of atopic dermatitis. J Dermatol Sci *74*, 229-235.

Kawai, T., and Akira, S. (2010). The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol *11*, 373-384.

Kawasaki, T., and Kawai, T. (2014). Toll-like receptor signaling pathways. Front Immunol *5*, 461.

Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K.D., and Gotz, F. (1988). Gallidermin: a new lanthionine-containing polypeptide antibiotic. Eur J Biochem *177*, 53-59.

Kennedy, A.D., and DeLeo, F.R. (2009). Neutrophil apoptosis and the resolution of infection. Immunol Res *43*, 25-61.

Kennedy, E.A., Connolly, J., Hourihane, J.O., Fallon, P.G., McLean, W.H.I., Murray, D., Jo, J.H., Segre, J.A., Kong, H.H., and Irvine, A.D. (2017). Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. J Allergy Clin Immunol *139*, 166-172.

Kenny, E.F., Herzig, A., Kruger, R., Muth, A., Mondal, S., Thompson, P.R., Brinkmann, V., Bernuth, H.V., and Zychlinsky, A. (2017). Diverse stimuli engage different neutrophil extracellular trap pathways. Elife *6*.

Khan, M.A., and Palaniyar, N. (2017). Transcriptional firing helps to drive NETosis. Sci Rep *7*, 41749.

Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J.S., Friday, S., Li, S., Patel, R.M., Subramanian, V.*, et al.* (2013). NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. Sci Transl Med *5*, 178ra140.

Kiekens, R.C., Thepen, T., Oosting, A.J., Bihari, I.C., Van De Winkel, J.G., Bruijnzeel-Koomen, C.A., and Knol, E.F. (2001). Heterogeneity within tissue-specific macrophage and dendritic cell populations during cutaneous inflammation in atopic dermatitis. Br J Dermatol *145*, 957-965.

Kim, B.S., Siracusa, M.C., Saenz, S.A., Noti, M., Monticelli, L.A., Sonnenberg, G.F., Hepworth, M.R., Van Voorhees, A.S., Comeau, M.R., and Artis, D. (2013). TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. Sci Transl Med *5*, 170ra116.

Kim, S., Kim, H.J., Yang, H.S., Kim, E., Huh, I.S., and Yang, J.M. (2011). IL-31 Serum Protein and Tissue mRNA Levels in Patients with Atopic Dermatitis. Ann Dermatol *23*, 468-473.

Kimber, I., Cumberbatch, M., Dearman, R.J., Bhushan, M., and Griffiths, C.E. (2000). Cytokines and chemokines in the initiation and regulation of epidermal Langerhans cell mobilization. Br J Dermatol *142*, 401-412.

Kinoshita, M., Kobayashi, N., Nagashima, S., Ishino, M., Otokozawa, S., Mise, K., Sumi, A., Tsutsumi, H., Uehara, N., Watanabe, N.*, et al.* (2008). Diversity of staphylocoagulase and identification of novel variants of staphylocoagulase gene in Staphylococcus aureus. Microbiol Immunol *52*, 334-348.

Kisich, K.O., Carspecken, C.W., Fieve, S., Boguniewicz, M., and Leung, D.Y. (2008). Defective killing of Staphylococcus aureus in atopic dermatitis is associated with reduced mobilization of human beta-defensin-3. J Allergy Clin Immunol *122*, 62-68.

Kloos, W.E., and Musselwhite, M.S. (1975). Distribution and persistence of Staphylococcus and Micrococcus species and other aerobic bacteria on human skin. Appl Microbiol *30*, 381-385.

Kobayashi, S.D., Malachowa, N., and DeLeo, F.R. (2017). Influence of Microbes on Neutrophil Life and Death. Front Cell Infect Microbiol *7*, 159.

Koch, S., Kohl, K., Klein, E., von Bubnoff, D., and Bieber, T. (2006). Skin homing of Langerhans cell precursors: adhesion, chemotaxis, and migration. J Allergy Clin Immunol *117*, 163-168.

Kolaczkowska, E., and Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol *13*, 159-175.

Kommineni, S., Bretl, D.J., Lam, V., Chakraborty, R., Hayward, M., Simpson, P., Cao, Y., Bousounis, P., Kristich, C.J., and Salzman, N.H. (2015). Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. Nature *526*, 719-722.

Kong, H.H., Oh, J., Deming, C., Conlan, S., Grice, E.A., Beatson, M.A., Nomicos, E., Polley, E.C., Komarow, H.D., Murray, P.R.*, et al.* (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res *22*, 850-859.

Kopf, M., Baumann, H., Freer, G., Freudenberg, M., Lamers, M., Kishimoto, T., Zinkernagel, R., Bluethmann, H., and Kohler, G. (1994). Impaired immune and acute-phase responses in interleukin-6-deficient mice. Nature *368*, 339-342.

Kostura, M.J., Tocci, M.J., Limjuco, G., Chin, J., Cameron, P., Hillman, A.G., Chartrain, N.A., and Schmidt, J.A. (1989). Identification of a monocyte specific pre-interleukin 1 beta convertase activity. Proc Natl Acad Sci U S A *86*, 5227-5231.

Koymans, K.J., Feitsma, L.J., Brondijk, T.H., Aerts, P.C., Lukkien, E., Lossl, P., van Kessel, K.P., de Haas, C.J., van Strijp, J.A., and Huizinga, E.G. (2015). Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3). Proceedings of the National Academy of Sciences of the United States of America *112*, 11018-11023.

Kretschmer, D., Gleske, A.K., Rautenberg, M., Wang, R., Koberle, M., Bohn, E., Schoneberg, T., Rabiet, M.J., Boulay, F., Klebanoff, S.J.*, et al.* (2010). Human formyl peptide receptor 2 senses highly pathogenic Staphylococcus aureus. Cell Host Microbe *7*, 463-473.

Krishna, S., and Miller, L.S. (2012). Host-pathogen interactions between the skin and Staphylococcus aureus. Curr Opin Microbiol *15*, 28-35.

Kumar, R., Jangir, P.K., Das, J., Taneja, B., and Sharma, R. (2017). Genome Analysis of Staphylococcus capitis TE8 Reveals Repertoire of Antimicrobial Peptides and Adaptation Strategies for Growth on Human Skin. Sci Rep *7*, 10447.

Kupper, T.S. (1990). Immune and inflammatory processes in cutaneous tissues. Mechanisms and speculations. J Clin Invest *86*, 1783-1789.

Kurokawa, K., Kim, M.S., Ichikawa, R., Ryu, K.H., Dohmae, N., Nakayama, H., and Lee, B.L. (2012). Environment-mediated accumulation of diacyl lipoproteins over their triacyl counterparts in Staphylococcus aureus. J Bacteriol *194*, 3299-3306.

Kurschus, F.C., and Moos, S. (2017). IL-17 for therapy. J Dermatol Sci *87*, 221-227.

Laborel-Preneron, E., Bianchi, P., Boralevi, F., Lehours, P., Fraysse, F., Morice-Picard, F., Sugai, M., Sato'o, Y., Badiou, C., Lina, G.*, et al.* (2015). 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*, e0141067.

Lai, Y., Cogen, A.L., Radek, K.A., Park, H.J., Macleod, D.T., Leichtle, A., Ryan, A.F., Di Nardo, A., and Gallo, R.L. (2010). Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. J Invest Dermatol *130*, 2211-2221.

Lai, Y., Di Nardo, A., Nakatsuji, T., Leichtle, A., Yang, Y., Cogen, A.L., Wu, Z.R., Hooper, L.V., Schmidt, R.R., von Aulock, S.*, et al.* (2009). Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. Nat Med *15*, 1377-1382.

Lai, Y., Villaruz, A.E., Li, M., Cha, D.J., Sturdevant, D.E., and Otto, M. (2007). The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci. Mol Microbiol *63*, 497-506.

Lamkanfi, M., and Dixit, V.M. (2014). Mechanisms and functions of inflammasomes. Cell *157*, 1013-1022.

Lande, R., Gregorio, J., Facchinetti, V., Chatterjee, B., Wang, Y.H., Homey, B., Cao, W., Wang, Y.H., Su, B., Nestle, F.O.*, et al.* (2007). Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature *449*, 564-569.

Le, K.Y., and Otto, M. (2015). Quorum-sensing regulation in staphylococci-an overview. Front Microbiol *6*, 1174.

Leung, D.Y., Harbeck, R., Bina, P., Reiser, R.F., Yang, E., Norris, D.A., Hanifin, J.M., and Sampson, H.A. (1993). Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. J Clin Invest *92*, 1374-1380.

Ley, R.E., Peterson, D.A., and Gordon, J.I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell *124*, 837-848.

Leyden, J.J., Marples, R.R., and Kligman, A.M. (1974). Staphylococcus aureus in the lesions of atopic dermatitis. Br J Dermatol *90*, 525-530.

Li, D., Lei, H., Li, Z., Li, H., Wang, Y., and Lai, Y. (2013). A novel lipopeptide from skin commensal activates TLR2/CD36-p38 MAPK signaling to increase antibacterial defense against bacterial infection. PLoS One *8*, e58288.

Li, D., Wang, W., Wu, Y., Ma, X., Zhou, W., and Lai, Y. (2019). Lipopeptide 78 from Staphylococcus epidermidis Activates beta-Catenin To Inhibit Skin Inflammation. J Immunol *202*, 1219-1228.

Li, J., Casanova, J.L., and Puel, A. (2018). Mucocutaneous IL-17 immunity in mice and humans: host defense vs. excessive inflammation. Mucosal Immunol *11*, 581-589.

Liang, S.C., Tan, X.Y., Luxenberg, D.P., Karim, R., Dunussi-Joannopoulos, K., Collins, M., and Fouser, L.A. (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med *203*, 2271-2279.

Lin, Z.Q., Kondo, T., Ishida, Y., Takayasu, T., and Mukaida, N. (2003). Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. J Leukoc Biol *73*, 713-721.

Linehan, J.L., Harrison, O.J., Han, S.J., Byrd, A.L., Vujkovic-Cvijin, I., Villarino, A.V., Sen, S.K., Shaik, J., Smelkinson, M., Tamoutounour, S.*, et al.* (2018). Non-classical Immunity Controls Microbiota Impact on Skin Immunity and Tissue Repair. Cell *172*, 784-796 e718.

Liu, F.T., Goodarzi, H., and Chen, H.Y. (2011). IgE, mast cells, and eosinophils in atopic dermatitis. Clin Rev Allergy Immunol *41*, 298-310.

Liu, H., Archer, N.K., Dillen, C.A., Wang, Y., Ashbaugh, A.G., Ortines, R.V., Kao, T., Lee, S.K., Cai, S.S., Miller, R.J.*, et al.* (2017). Staphylococcus aureus Epicutaneous Exposure Drives Skin Inflammation via IL-36-Mediated T Cell Responses. Cell Host Microbe *22*, 653-666 e655.

Lowy, F.D. (1998). Staphylococcus aureus infections. N Engl J Med *339*, 520-532.

Luo, Y., McLean, D.T., Linden, G.J., McAuley, D.F., McMullan, R., and Lundy, F.T. (2017). The Naturally Occurring Host Defense Peptide, LL-37, and Its Truncated Mimetics KE-18 and KR-12 Have Selected Biocidal and Antibiofilm Activities Against Candida albicans, Staphylococcus aureus, and Escherichia coli In vitro. Front Microbiol *8*, 544.

Maher, S., and McClean, S. (2006). Investigation of the cytotoxicity of eukaryotic and prokaryotic antimicrobial peptides in intestinal epithelial cells in vitro. Biochem Pharmacol *71*, 1289-1298.

Malachowa, N., Kobayashi, S.D., Freedman, B., Dorward, D.W., and DeLeo, F.R. (2013). Staphylococcus aureus leukotoxin GH promotes formation of neutrophil extracellular traps. J Immunol *191*, 6022-6029.

Malhotra, N., Yoon, J., Leyva-Castillo, J.M., Galand, C., Archer, N., Miller, L.S., and Geha, R.S. (2016). IL-22 derived from gammadelta T cells restricts Staphylococcus aureus infection of mechanically injured skin. J Allergy Clin Immunol *138*, 1098-1107 e1093.

Mantovani, A., Biswas, S.K., Galdiero, M.R., Sica, A., and Locati, M. (2013). Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol *229*, 176-185.

Martin, C.J., Booty, M.G., Rosebrock, T.R., Nunes-Alves, C., Desjardins, D.M., Keren, I., Fortune, S.M., Remold, H.G., and Behar, S.M. (2012). Efferocytosis is an innate antibacterial mechanism. Cell Host Microbe *12*, 289-300.

Martin, H., Laborel-Preneron, E., Fraysse, F., Nguyen, T., Schmitt, A.M., Redoules, D., and Davrinche, C. (2016). Aquaphilus dolomiae extract counteracts the effects of cutaneous S. aureus secretome isolated from atopic children on CD4(+) T cell activation. Pharm Biol *54*, 2782-2785.

Meisel, J.S., Sfyroera, G., Bartow-McKenney, C., Gimblet, C., Bugayev, J., Horwinski, J., Kim, B., Brestoff, J.R., Tyldsley, A.S., Zheng, Q.*, et al.* (2018). Commensal microbiota modulate gene expression in the skin. Microbiome *6*, 20.

Merad, M., Sathe, P., Helft, J., Miller, J., and Mortha, A. (2013). The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol *31*, 563-604.

Meric, G., Mageiros, L., Pensar, J., Laabei, M., Yahara, K., Pascoe, B., Kittiwan, N., Tadee, P., Post, V., Lamble, S.*, et al.* (2018). Disease-associated genotypes of the commensal skin bacterium Staphylococcus epidermidis. Nat Commun *9*, 5034.

Metzler, K.D., Goosmann, C., Lubojemska, A., Zychlinsky, A., and Papayannopoulos, V. (2014). A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis. Cell Rep *8*, 883-896.

Meylan, P., Lang, C., Mermoud, S., Johannsen, A., Norrenberg, S., Hohl, D., Vial, Y., Prod'hom, G., Greub, G., Kypriotou, M.*, et al.* (2017). Skin Colonization by Staphylococcus aureus Precedes the Clinical Diagnosis of Atopic Dermatitis in Infancy. J Invest Dermatol *137*, 2497-2504.

Mildner, A., and Jung, S. (2014). Development and function of dendritic cell subsets. Immunity *40*, 642-656.

Miller, L.S., and Modlin, R.L. (2007). Toll-like receptors in the skin. Semin Immunopathol *29*, 15-26.

Miller, L.S., O'Connell, R.M., Gutierrez, M.A., Pietras, E.M., Shahangian, A., Gross, C.E., Thirumala, A., Cheung, A.L., Cheng, G., and Modlin, R.L. (2006). MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity *24*, 79-91.

Miller, L.S., Pietras, E.M., Uricchio, L.H., Hirano, K., Rao, S., Lin, H., O'Connell, R.M., Iwakura, Y., Cheung, A.L., Cheng, G.*, et al.* (2007). Inflammasome-mediated production of IL-1beta is required for neutrophil recruitment against Staphylococcus aureus in vivo. J Immunol *179*, 6933-6942.

Molne, L., Verdrengh, M., and Tarkowski, A. (2000). Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus. Infect Immun *68*, 6162-6167.

Moos, S., Mohebiany, A.N., Waisman, A., and Kurschus, F.C. (2019). Imiquimod-Induced Psoriasis in Mice Depends on the IL-17 Signaling of Keratinocytes. J Invest Dermatol *139*, 1110-1117.

Mora, J.R., Cheng, G., Picarella, D., Briskin, M., Buchanan, N., and von Andrian, U.H. (2005). Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. J Exp Med *201*, 303-316.

Mueller, S.N., Gebhardt, T., Carbone, F.R., and Heath, W.R. (2013). Memory T cell subsets, migration patterns, and tissue residence. Annu Rev Immunol *31*, 137-161.

Muller, A., Ulm, H., Reder-Christ, K., Sahl, H.G., and Schneider, T. (2012). Interaction of type A lantibiotics with undecaprenol-bound cell envelope precursors. Microb Drug Resist *18*, 261-270.

Munzenmayer, L., Geiger, T., Daiber, E., Schulte, B., Autenrieth, S.E., Fraunholz, M., and Wolz, C. (2016). Influence of Sae-regulated and Agr-regulated factors on the escape of Staphylococcus aureus from human macrophages. Cell Microbiol *18*, 1172-1183.

Murphy, J.E., Robert, C., and Kupper, T.S. (2000). Interleukin-1 and cutaneous inflammation: a crucial link between innate and acquired immunity. J Invest Dermatol *114*, 602-608.

Myles, I.A., Earland, N.J., Anderson, E.D., Moore, I.N., Kieh, M.D., Williams, K.W., Saleem, A., Fontecilla, N.M., Welch, P.A., Darnell, D.A.*, et al.* (2018). First-in-human topical microbiome transplantation with Roseomonas mucosa for atopic dermatitis. JCI Insight *3*.

Nagao, K., Ginhoux, F., Leitner, W.W., Motegi, S., Bennett, C.L., Clausen, B.E., Merad, M., and Udey, M.C. (2009). Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. Proc Natl Acad Sci U S A *106*, 3312-3317.

Nagao, K., Kobayashi, T., Moro, K., Ohyama, M., Adachi, T., Kitashima, D.Y., Ueha, S., Horiuchi, K., Tanizaki, H., Kabashima, K.*, et al.* (2012). Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. Nat Immunol *13*, 744-752.

Naik, S., Bouladoux, N., Linehan, J.L., Han, S.J., Harrison, O.J., Wilhelm, C., Conlan, S., Himmelfarb, S., Byrd, A.L., Deming, C.*, et al.* (2015). Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. Nature *520*, 104-108.

Naik, S., Bouladoux, N., Wilhelm, C., Molloy, M.J., Salcedo, R., Kastenmuller, W., Deming, C., Quinones, M., Koo, L., Conlan, S.*, et al.* (2012). Compartmentalized control of skin immunity by resident commensals. Science *337*, 1115-1119.

Nakagawa, S., Matsumoto, M., Katayama, Y., Oguma, R., Wakabayashi, S., Nygaard, T., Saijo, S., Inohara, N., Otto, M., Matsue, H.*, et al.* (2017). Staphylococcus aureus Virulent PSMalpha Peptides Induce Keratinocyte Alarmin Release to Orchestrate IL-17-Dependent Skin Inflammation. Cell Host Microbe *22*, 667-677 e665.

Nakamura, Y., Oscherwitz, J., Cease, K.B., Chan, S.M., Munoz-Planillo, R., Hasegawa, M., Villaruz, A.E., Cheung, G.Y., McGavin, M.J., Travers, J.B.*, et al.*

(2013). Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. Nature *503*, 397-401.

Nakatsuji, T., Chen, T.H., Butcher, A.M., Trzoss, L.L., Nam, S.J., Shirakawa, K.T., Zhou, W., Oh, J., Otto, M., Fenical, W.*, et al.* (2018). A commensal strain of Staphylococcus epidermidis protects against skin neoplasia. Sci Adv *4*, eaao4502.

Nakatsuji, T., Chen, T.H., Narala, S., Chun, K.A., Two, A.M., Yun, T., Shafiq, F., Kotol, P.F., Bouslimani, A., Melnik, A.V.*, et al.* (2017). Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med *9*.

Nakatsuji, T., Chen, T.H., Two, A.M., Chun, K.A., Narala, S., Geha, R.S., Hata, T.R., and Gallo, R.L. (2016). Staphylococcus aureus Exploits Epidermal Barrier Defects in Atopic Dermatitis to Trigger Cytokine Expression. J Invest Dermatol *136*, 2192-2200.

Naqvi, N., Ahuja, K., Selvapandiyan, A., Dey, R., Nakhasi, H., and Puri, N. (2017). Role of Mast Cells in clearance of Leishmania through extracellular trap formation. Sci Rep *7*, 13240.

Needham, G.M., Ferris, V., and Spink, W.W. (1945). The correlation of the rapid slide method with the tube method for differentiating coagulase-positive from coagulase-negative strains of staphylococci. Am J Clin Pathol *9*, 83-85.

Neeli, I., Khan, S.N., and Radic, M. (2008). Histone deimination as a response to inflammatory stimuli in neutrophils. J Immunol *180*, 1895-1902.

Neis, M.M., Peters, B., Dreuw, A., Wenzel, J., Bieber, T., Mauch, C., Krieg, T., Stanzel, S., Heinrich, P.C., Merk, H.F.*, et al.* (2006). Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. J Allergy Clin Immunol *118*, 930-937.

Nestle, F.O., Di Meglio, P., Qin, J.Z., and Nickoloff, B.J. (2009). Skin immune sentinels in health and disease. Nat Rev Immunol *9*, 679-691.

Neveu, W.A., Allard, J.B., Dienz, O., Wargo, M.J., Ciliberto, G., Whittaker, L.A., and Rincon, M. (2009). IL-6 is required for airway mucus production induced by inhaled fungal allergens. J Immunol *183*, 1732-1738.

Nguyen, M.T., and Gotz, F. (2016). Lipoproteins of Gram-Positive Bacteria: Key Players in the Immune Response and Virulence. Microbiol Mol Biol Rev *80*, 891-903.

Nguyen, M.T., Hanzelmann, D., Hartner, T., Peschel, A., and Gotz, F. (2016). Skin-Specific Unsaturated Fatty Acids Boost the Staphylococcus aureus Innate Immune Response. Infect Immun *84*, 205-215.

Nguyen, M.T., Luqman, A., Bitschar, K., Hertlein, T., Dick, J., Ohlsen, K., Broker, B., Schittek, B., and Gotz, F. (2017). Staphylococcal (phospho)lipases promote biofilm formation and host cell invasion. Int J Med Microbiol.

Niemirowicz, K., Piktel, E., Wilczewska, A.Z., Markiewicz, K.H., Durnas, B., Watek, M., Puszkarz, I., Wroblewska, M., Niklinska, W., Savage, P.B.*, et al.* (2016). Core-shell magnetic nanoparticles display synergistic antibacterial effects against

Pseudomonas aeruginosa and Staphylococcus aureus when combined with cathelicidin LL-37 or selected ceragenins. Int J Nanomedicine *11*, 5443-5455.

Niyonsaba, F., Kiatsurayanon, C., Chieosilapatham, P., and Ogawa, H. (2017). Friends or Foes? Host defense (antimicrobial) peptides and proteins in human skin diseases. Exp Dermatol *26*, 989-998.

Niyonsaba, F., Nagaoka, I., Ogawa, H., and Okumura, K. (2009a). Multifunctional antimicrobial proteins and peptides: natural activators of immune systems. Curr Pharm Des *15*, 2393-2413.

Niyonsaba, F., Suzuki, A., Ushio, H., Nagaoka, I., Ogawa, H., and Okumura, K. (2009b). The human antimicrobial peptide dermcidin activates normal human keratinocytes. Br J Dermatol *160*, 243-249.

Nomura, I., Goleva, E., Howell, M.D., Hamid, Q.A., Ong, P.Y., Hall, C.F., Darst, M.A., Gao, B., Boguniewicz, M., Travers, J.B.*, et al.* (2003). Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. J Immunol *171*, 3262-3269.

Nosbaum, A., Prevel, N., Truong, H.A., Mehta, P., Ettinger, M., Scharschmidt, T.C., Ali, N.H., Pauli, M.L., Abbas, A.K., and Rosenblum, M.D. (2016). Cutting Edge: Regulatory T Cells Facilitate Cutaneous Wound Healing. J Immunol *196*, 2010-2014.

Nourshargh, S., and Alon, R. (2014). Leukocyte migration into inflamed tissues. Immunity *41*, 694-707.

Novick, R.P., and Geisinger, E. (2008). Quorum sensing in staphylococci. Annu Rev Genet *42*, 541-564.

Oh, J., Byrd, A.L., Deming, C., Conlan, S., Program, N.C.S., Kong, H.H., and Segre, J.A. (2014). Biogeography and individuality shape function in the human skin metagenome. Nature *514*, 59-64.

Oh, J., Byrd, A.L., Park, M., Program, N.C.S., Kong, H.H., and Segre, J.A. (2016). Temporal Stability of the Human Skin Microbiome. Cell *165*, 854-866.

Oh, J., Conlan, S., Polley, E.C., Segre, J.A., and Kong, H.H. (2012). Shifts in human skin and nares microbiota of healthy children and adults. Genome Med *4*, 77.

Okubo, K., Kurosawa, M., Kamiya, M., Urano, Y., Suzuki, A., Yamamoto, K., Hase, K., Homma, K., Sasaki, J., Miyauchi, H.*, et al.* (2018). Macrophage extracellular trap formation promoted by platelet activation is a key mediator of rhabdomyolysis-induced acute kidney injury. Nat Med *24*, 232-238.

Ong, P.Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., Gallo, R.L., and Leung, D.Y. (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. N Engl J Med *347*, 1151-1160.

Onoue, A., Kabashima, K., Kobayashi, M., Mori, T., and Tokura, Y. (2009). Induction of eosinophil- and Th2-attracting epidermal chemokines and cutaneous late-phase reaction in tape-stripped skin. Exp Dermatol *18*, 1036-1043.

Otto, M. (2009a). Bacterial sensing of antimicrobial peptides. Contrib Microbiol *16*, 136-149.

Otto, M. (2009b). Staphylococcus epidermidis--the 'accidental' pathogen. Nat Rev Microbiol *7*, 555-567.

Otto, M. (2010). Staphylococcus colonization of the skin and antimicrobial peptides. Expert Rev Dermatol *5*, 183-195.

Otto, M. (2014). Staphylococcus aureus toxins. Curr Opin Microbiol *17*, 32-37.

Otto, M., Echner, H., Voelter, W., and Gotz, F. (2001). Pheromone cross-inhibition between Staphylococcus aureus and Staphylococcus epidermidis. Infect Immun *69*, 1957-1960.

Otto, M., Sussmuth, R., Vuong, C., Jung, G., and Gotz, F. (1999). Inhibition of virulence factor expression in Staphylococcus aureus by the Staphylococcus epidermidis agr pheromone and derivatives. FEBS Lett *450*, 257-262.

Overhage, J., Campisano, A., Bains, M., Torfs, E.C., Rehm, B.H., and Hancock, R.E. (2008). Human host defense peptide LL-37 prevents bacterial biofilm formation. Infect Immun *76*, 4176-4182.

Oyoshi, M.K., Larson, R.P., Ziegler, S.F., and Geha, R.S. (2010). Mechanical injury polarizes skin dendritic cells to elicit a T(H)2 response by inducing cutaneous thymic stromal lymphopoietin expression. J Allergy Clin Immunol *126*, 976-984, 984 e971-975.

Paharik, A.E., Parlet, C.P., Chung, N., Todd, D.A., Rodriguez, E.I., Van Dyke, M.J., Cech, N.B., and Horswill, A.R. (2017). Coagulase-Negative Staphylococcal Strain Prevents Staphylococcus aureus Colonization and Skin Infection by Blocking Quorum Sensing. Cell Host Microbe *22*, 746-756 e745.

Pamer, E.G. (2016). Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens. Science *352*, 535-538.

Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol *18*, 134-147.

Papayannopoulos, V., Metzler, K.D., Hakkim, A., and Zychlinsky, A. (2010). Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol *191*, 677-691.

Papayannopoulos, V., and Zychlinsky, A. (2009). NETs: a new strategy for using old weapons. Trends Immunol *30*, 513-521.

Pasparakis, M., Haase, I., and Nestle, F.O. (2014). Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol *14*, 289-301.

Patti, J.M., Allen, B.L., McGavin, M.J., and Hook, M. (1994). MSCRAMM-mediated adherence of microorganisms to host tissues. Annu Rev Microbiol *48*, 585-617.

Paulmann, M., Arnold, T., Linke, D., Ozdirekcan, S., Kopp, A., Gutsmann, T., Kalbacher, H., Wanke, I., Schuenemann, V.J., Habeck, M.*, et al.* (2012). Structure-activity analysis of the dermcidin-derived peptide DCD-1L, an anionic antimicrobial peptide present in human sweat. J Biol Chem *287*, 8434-8443.

Perez-Lopez, A., Behnsen, J., Nuccio, S.P., and Raffatellu, M. (2016). Mucosal immunity to pathogenic intestinal bacteria. Nat Rev Immunol *16*, 135-148.

Peschel, A., and Otto, M. (2013). Phenol-soluble modulins and staphylococcal infection. Nature reviews Microbiology *11*, 667-673.

Peschel, A., and Sahl, H.G. (2006). The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nature reviews Microbiology *4*, 529-536.

Pilsczek, F.H., Salina, D., Poon, K.K., Fahey, C., Yipp, B.G., Sibley, C.D., Robbins, S.M., Green, F.H., Surette, M.G., Sugai, M.*, et al.* (2010). A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol *185*, 7413-7425.

Planet, P.J. (2017). Life After USA300: The Rise and Fall of a Superbug. J Infect Dis *215*, S71-S77.

Proctor, R.A. (2012). Challenges for a universal Staphylococcus aureus vaccine. Clin Infect Dis *54*, 1179-1186.

Puel, A., Picard, C., Lorrot, M., Pons, C., Chrabieh, M., Lorenzo, L., Mamani-Matsuda, M., Jouanguy, E., Gendrel, D., and Casanova, J.L. (2008). Recurrent staphylococcal cellulitis and subcutaneous abscesses in a child with autoantibodies against IL-6. J Immunol *180*, 647-654.

Rademacher, F., Simanski, M., Hesse, B., Dombrowsky, G., Vent, N., Glaser, R., and Harder, J. (2019). Staphylococcus epidermidis Activates Aryl Hydrocarbon Receptor Signaling in Human Keratinocytes: Implications for Cutaneous Defense. J Innate Immun *11*, 125-135.

Rahman, M.M., and McFadden, G. (2011). Modulation of NF-kappaB signalling by microbial pathogens. Nat Rev Microbiol *9*, 291-306.

Rajarathnam, K., Schnoor, M., Richardson, R.M., and Rajagopal, S. (2019). How do chemokines navigate neutrophils to the target site: Dissecting the structural mechanisms and signaling pathways. Cell Signal *54*, 69-80.

Ramirez-Carrozzi, V., Sambandam, A., Luis, E., Lin, Z., Jeet, S., Lesch, J., Hackney, J., Kim, J., Zhou, M., Lai, J.*, et al.* (2011). IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. Nat Immunol *12*, 1159-1166.

Ridaura, V.K., Bouladoux, N., Claesen, J., Chen, Y.E., Byrd, A.L., Constantinides, M.G., Merrill, E.D., Tamoutounour, S., Fischbach, M.A., and Belkaid, Y. (2018). Contextual control of skin immunity and inflammation by Corynebacterium. J Exp Med *215*, 785-799.

Rieg, S., Garbe, C., Sauer, B., Kalbacher, H., and Schittek, B. (2004). Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions. Br J Dermatol *151*, 534-539.

Rieg, S., Steffen, H., Seeber, S., Humeny, A., Kalbacher, H., Dietz, K., Garbe, C., and Schittek, B. (2005). Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. J Immunol *174*, 8003-8010.

Ring, J., Kramer, U., Schafer, T., and Behrendt, H. (2001). Why are allergies increasing? Curr Opin Immunol *13*, 701-708.

Rojo, A., Aguinaga, A., Monecke, S., Yuste, J.R., Gastaminza, G., and Espana, A. (2014). Staphylococcus aureus genomic pattern and atopic dermatitis: may factors other than superantigens be involved? Eur J Clin Microbiol Infect Dis *33*, 651-658.

Rot, A., and von Andrian, U.H. (2004). Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu Rev Immunol *22*, 891-928.

Roth, S.A., Simanski, M., Rademacher, F., Schroder, L., and Harder, J. (2014). The pattern recognition receptor NOD2 mediates Staphylococcus aureus-induced IL-17C expression in keratinocytes. J Invest Dermatol *134*, 374-380.

Ruddy, M.J., Wong, G.C., Liu, X.K., Yamamoto, H., Kasayama, S., Kirkwood, K.L., and Gaffen, S.L. (2004). Functional cooperation between interleukin-17 and tumor necrosis factor-alpha is mediated by CCAAT/enhancer-binding protein family members. J Biol Chem *279*, 2559-2567.

Ryu, S., Song, P.I., Seo, C.H., Cheong, H., and Park, Y. (2014). Colonization and infection of the skin by S. aureus: immune system evasion and the response to cationic antimicrobial peptides. Int J Mol Sci *15*, 8753-8772.

Sabat, R., Wolk, K., Loyal, L., Docke, W.D., and Ghoreschi, K. (2019). T cell pathology in skin inflammation. Semin Immunopathol.

Saffarzadeh, M., Juenemann, C., Queisser, M.A., Lochnit, G., Barreto, G., Galuska, S.P., Lohmeyer, J., and Preissner, K.T. (2012). Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One *7*, e32366.

Sakaguchi, S. (2005). Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol *6*, 345-352.

Salamero, J., Bausinger, H., Mommaas, A.M., Lipsker, D., Proamer, F., Cazenave, J.P., Goud, B., de la Salle, H., and Hanau, D. (2001). CD1a molecules traffic through the early recycling endosomal pathway in human Langerhans cells. J Invest Dermatol *116*, 401-408.

Salimi, M., Barlow, J.L., Saunders, S.P., Xue, L., Gutowska-Owsiak, D., Wang, X., Huang, L.C., Johnson, D., Scanlon, S.T., McKenzie, A.N.*, et al.* (2013). A role for IL-

25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. J Exp Med *210*, 2939-2950.

SanMiguel, A., and Grice, E.A. (2015). Interactions between host factors and the skin microbiome. Cell Mol Life Sci *72*, 1499-1515.

Sassone-Corsi, M., and Raffatellu, M. (2015). No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens. J Immunol *194*, 4081-4087.

Sato, M., Sawamura, D., Ina, S., Yaguchi, T., Hanada, K., and Hashimoto, I. (1999). In vivo introduction of the interleukin 6 gene into human keratinocytes: induction of epidermal proliferation by the fully spliced form of interleukin 6, but not by the alternatively spliced form. Arch Dermatol Res *291*, 400-404.

Scharschmidt, T.C., and Fischbach, M.A. (2013). What Lives On Our Skin: Ecology, Genomics and Therapeutic Opportunities Of the Skin Microbiome. Drug Discov Today Dis Mech *10*.

Scharschmidt, T.C., Vasquez, K.S., Pauli, M.L., Leitner, E.G., Chu, K., Truong, H.A., Lowe, M.M., Sanchez Rodriguez, R., Ali, N., Laszik, Z.G.*, et al.* (2017). Commensal Microbes and Hair Follicle Morphogenesis Coordinately Drive Treg Migration into Neonatal Skin. Cell Host Microbe *21*, 467-477 e465.

Scharschmidt, T.C., Vasquez, K.S., Truong, H.A., Gearty, S.V., Pauli, M.L., Nosbaum, A., Gratz, I.K., Otto, M., Moon, J.J., Liese, J.*, et al.* (2015). A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes. Immunity *43*, 1011-1021.

Schilling, N.A., Berscheid, A., Schumacher, J., Saur, J.S., Konnerth, M.C., Wirtz, S.N., Beltran-Belena, J.M., Zipperer, A., Krismer, B., Peschel, A.*, et al.* (2019). Synthetic Lugdunin Analogues Reveal Essential Structural Motifs for Antimicrobial Action and Proton Translocation Capability. Angew Chem Int Ed Engl *58*, 9234-9238.

Schittek, B. (2011). The antimicrobial skin barrier in patients with atopic dermatitis. Curr Probl Dermatol *41*, 54-67.

Schittek, B., Hipfel, R., Sauer, B., Bauer, J., Kalbacher, H., Stevanovic, S., Schirle, M., Schroeder, K., Blin, N., Meier, F.*, et al.* (2001). Dermcidin: a novel human antibiotic peptide secreted by sweat glands. Nat Immunol *2*, 1133-1137.

Schittek, B., Paulmann, M., Senyurek, I., and Steffen, H. (2008). The role of antimicrobial peptides in human skin and in skin infectious diseases. Infect Disord Drug Targets *8*, 135-143.

Schleifer, K.H., and Kloos, W.E. (1975). A simple test system for the separation of staphylococci from micrococci. J Clin Microbiol *1*, 337-338.

Schloissnig, S., Arumugam, M., Sunagawa, S., Mitreva, M., Tap, J., Zhu, A., Waller, A., Mende, D.R., Kultima, J.R., Martin, J.*, et al.* (2013). Genomic variation landscape of the human gut microbiome. Nature *493*, 45-50.

Schnell, N., Entian, K.D., Schneider, U., Gotz, F., Zahner, H., Kellner, R., and Jung, G. (1988). Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. Nature *333*, 276-278.

Schroder, J.M., and Harder, J. (2006). Antimicrobial skin peptides and proteins. Cell Mol Life Sci *63*, 469-486.

Schwab, U., Gilligan, P., Jaynes, J., and Henke, D. (1999). In vitro activities of designed antimicrobial peptides against multidrug-resistant cystic fibrosis pathogens. Antimicrob Agents Chemother *43*, 1435-1440.

Segal, A.W. (2005). How neutrophils kill microbes. Annu Rev Immunol *23*, 197-223.

Segre, J.A. (2006). Epidermal barrier formation and recovery in skin disorders. J Clin Invest *116*, 1150-1158.

Sehra, S., Yao, Y., Howell, M.D., Nguyen, E.T., Kansas, G.S., Leung, D.Y., Travers, J.B., and Kaplan, M.H. (2010). IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. J Immunol *184*, 3186-3190.

Sere, K., Baek, J.H., Ober-Blobaum, J., Muller-Newen, G., Tacke, F., Yokota, Y., Zenke, M., and Hieronymus, T. (2012). Two distinct types of Langerhans cells populate the skin during steady state and inflammation. Immunity *37*, 905-916.

Sieprawska-Lupa, M., Mydel, P., Krawczyk, K., Wojcik, K., Puklo, M., Lupa, B., Suder, P., Silberring, J., Reed, M., Pohl, J.*, et al.* (2004). Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. Antimicrob Agents Chemother *48*, 4673-4679.

Simanski, M., Dressel, S., Glaser, R., and Harder, J. (2010). RNase 7 protects healthy skin from Staphylococcus aureus colonization. J Invest Dermatol *130*, 2836-2838.

Sivan, A., Corrales, L., Hubert, N., Williams, J.B., Aquino-Michaels, K., Earley, Z.M., Benyamin, F.W., Lei, Y.M., Jabri, B., Alegre, M.L.*, et al.* (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science *350*, 1084-1089.

Skabytska, Y., Kaesler, S., Volz, T., and Biedermann, T. (2016). The role of innate immune signaling in the pathogenesis of atopic dermatitis and consequences for treatments. Semin Immunopathol *38*, 29-43.

Skabytska, Y., Wolbing, F., Gunther, C., Koberle, M., Kaesler, S., Chen, K.M., Guenova, E., Demircioglu, D., Kempf, W.E., Volz, T.*, et al.* (2014). Cutaneous innate immune sensing of Toll-like receptor 2-6 ligands suppresses T cell immunity by inducing myeloid-derived suppressor cells. Immunity *41*, 762-775.

Skov, L., Olsen, J.V., Giorno, R., Schlievert, P.M., Baadsgaard, O., and Leung, D.Y. (2000). Application of Staphylococcal enterotoxin B on normal and atopic skin induces up-regulation of T cells by a superantigen-mediated mechanism. J Allergy Clin Immunol *105*, 820-826.

Sparber, F. (2014). Langerhans cells: an update. J Dtsch Dermatol Ges *12*, 1107-1111.

Spaulding, A.R., Salgado-Pabon, W., Kohler, P.L., Horswill, A.R., Leung, D.Y., and Schlievert, P.M. (2013). Staphylococcal and streptococcal superantigen exotoxins. Clinical microbiology reviews *26*, 422-447.

Stacy, A., and Belkaid, Y. (2019). Microbial guardians of skin health. Science *363*, 227-228.

Stossel, H., Koch, F., Kampgen, E., Stoger, P., Lenz, A., Heufler, C., Romani, N., and Schuler, G. (1990). Disappearance of certain acidic organelles (endosomes and Langerhans cell granules) accompanies loss of antigen processing capacity upon culture of epidermal Langerhans cells. J Exp Med *172*, 1471-1482.

Sugimoto, S., Iwamoto, T., Takada, K., Okuda, K., Tajima, A., Iwase, T., and Mizunoe, Y. (2013). Staphylococcus epidermidis Esp degrades specific proteins associated with Staphylococcus aureus biofilm formation and host-pathogen interaction. J Bacteriol *195*, 1645-1655.

Surewaard, B.G., de Haas, C.J., Vervoort, F., Rigby, K.M., DeLeo, F.R., Otto, M., van Strijp, J.A., and Nijland, R. (2013). Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. Cell Microbiol *15*, 1427-1437.

Takahashi, T., Kimura, Y., Niwa, K., Ohmiya, Y., Fujimura, T., Yamasaki, K., and Aiba, S. (2013). In vivo imaging demonstrates ATP release from murine keratinocytes and its involvement in cutaneous inflammation after tape stripping. J Invest Dermatol *133*, 2407-2415.

Takeuchi, O., Hoshino, K., and Akira, S. (2000). Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection. J Immunol *165*, 5392-5396.

Tan, S.Y., Roediger, B., and Weninger, W. (2015). The role of chemokines in cutaneous immunosurveillance. Immunol Cell Biol *93*, 337-346.

Tauber, M., Balica, S., Hsu, C.Y., Jean-Decoster, C., Lauze, C., Redoules, D., Viode, C., Schmitt, A.M., Serre, G., Simon, M.*, et al.* (2016). Staphylococcus aureus density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. J Allergy Clin Immunol *137*, 1272-1274 e1273.

Tawaratsumida, K., Furuyashiki, M., Katsumoto, M., Fujimoto, Y., Fukase, K., Suda, Y., and Hashimoto, M. (2009). Characterization of N-terminal structure of TLR2-activating lipoprotein in Staphylococcus aureus. J Biol Chem *284*, 9147-9152.

Thammavongsa, V., Missiakas, D.M., and Schneewind, O. (2013). Staphylococcus aureus degrades neutrophil extracellular traps to promote immune cell death. Science *342*, 863-866.

Thoendel, M., Kavanaugh, J.S., Flack, C.E., and Horswill, A.R. (2011). Peptide signaling in the staphylococci. Chem Rev *111*, 117-151.

Thornberry, N.A., Bull, H.G., Calaycay, J.R., Chapman, K.T., Howard, A.D., Kostura, M.J., Miller, D.K., Molineaux, S.M., Weidner, J.R., Aunins, J.*, et al.* (1992). A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature *356*, 768-774.

Tomura, M., Hata, A., Matsuoka, S., Shand, F.H., Nakanishi, Y., Ikebuchi, R., Ueha, S., Tsutsui, H., Inaba, K., Matsushima, K.*, et al.* (2014). Tracking and quantification of dendritic cell migration and antigen trafficking between the skin and lymph nodes. Sci Rep *4*, 6030.

Totte, J.E., van der Feltz, W.T., Hennekam, M., van Belkum, A., van Zuuren, E.J., and Pasmans, S.G. (2016). Prevalence and odds of Staphylococcus aureus carriage in atopic dermatitis: a systematic review and meta-analysis. Br J Dermatol *175*, 687-695.

Travers, J.B., Kozman, A., Mousdicas, N., Saha, C., Landis, M., Al-Hassani, M., Yao, W., Yao, Y., Hyatt, A.M., Sheehan, M.P.*, et al.* (2010). Infected atopic dermatitis lesions contain pharmacologic amounts of lipoteichoic acid. J Allergy Clin Immunol *125*, 146-152 e141-142.

Turner, N.A., Sharma-Kuinkel, B.K., Maskarinec, S.A., Eichenberger, E.M., Shah, P.P., Carugati, M., Holland, T.L., and Fowler, V.G., Jr. (2019). Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. Nat Rev Microbiol *17*, 203-218.

Urban, C.F., Ermert, D., Schmid, M., Abu-Abed, U., Goosmann, C., Nacken, W., Brinkmann, V., Jungblut, P.R., and Zychlinsky, A. (2009). Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog *5*, e1000639.

Valladeau, J., Ravel, O., Dezutter-Dambuyant, C., Moore, K., Kleijmeer, M., Liu, Y., Duvert-Frances, V., Vincent, C., Schmitt, D., Davoust, J.*, et al.* (2000). Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. Immunity *12*, 71-81.

van der Poll, T., Keogh, C.V., Guirao, X., Buurman, W.A., Kopf, M., and Lowry, S.F. (1997). Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. J Infect Dis *176*, 439-444.

van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., Visser, C.E., Kuijper, E.J., Bartelsman, J.F., Tijssen, J.G.*, et al.* (2013). Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med *368*, 407-415.

Vandeghinste, N., Klattig, J., Jagerschmidt, C., Lavazais, S., Marsais, F., Haas, J.D., Auberval, M., Lauffer, F., Moran, T., Ongenaert, M.*, et al.* (2018). Neutralization of IL-17C Reduces Skin Inflammation in Mouse Models of Psoriasis and Atopic Dermatitis. J Invest Dermatol *138*, 1555-1563.

Varol, C., Mildner, A., and Jung, S. (2015). Macrophages: development and tissue specialization. Annu Rev Immunol *33*, 643-675.

Verdrengh, M., and Tarkowski, A. (1997). Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus. Infect Immun *65*, 2517-2521.

Vetizou, M., Pitt, J.M., Daillere, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M.P., Duong, C.P.*, et al.* (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science *350*, 1079-1084.

Villanueva, E., Yalavarthi, S., Berthier, C.C., Hodgin, J.B., Khandpur, R., Lin, A.M., Rubin, C.J., Zhao, W., Olsen, S.H., Klinker, M.*, et al.* (2011). Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol *187*, 538-552.

Visser, M.J.E., Kell, D.B., and Pretorius, E. (2019). Bacterial Dysbiosis and Translocation in Psoriasis Vulgaris. Front Cell Infect Microbiol *9*, 7.

Vivier, E., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J.P., Eberl, G., Koyasu, S., Locksley, R.M., McKenzie, A.N.J., Mebius, R.E.*, et al.* (2018). Innate Lymphoid Cells: 10 Years On. Cell *174*, 1054-1066.

Volz, T., Kaesler, S., Draing, C., Hartung, T., Rocken, M., Skabytska, Y., and Biedermann, T. (2018). Induction of IL-10-balanced immune profiles following exposure to LTA from Staphylococcus epidermidis. Exp Dermatol *27*, 318-326.

Volz, T., Nega, M., Buschmann, J., Kaesler, S., Guenova, E., Peschel, A., Rocken, M., Gotz, F., and Biedermann, T. (2010). Natural Staphylococcus aureus-derived peptidoglycan fragments activate NOD2 and act as potent costimulators of the innate immune system exclusively in the presence of TLR signals. FASEB J *24*, 4089-4102.

Volz, T., Skabytska, Y., Guenova, E., Chen, K.M., Frick, J.S., Kirschning, C.J., Kaesler, S., Rocken, M., and Biedermann, T. (2014). Nonpathogenic bacteria alleviating atopic dermatitis inflammation induce IL-10-producing dendritic cells and regulatory Tr1 cells. J Invest Dermatol *134*, 96-104.

von Eiff, C., Becker, K., Machka, K., Stammer, H., and Peters, G. (2001). Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med *344*, 11-16.

von Kockritz-Blickwede, M., Goldmann, O., Thulin, P., Heinemann, K., Norrby-Teglund, A., Rohde, M., and Medina, E. (2008). Phagocytosis-independent antimicrobial activity of mast cells by means of extracellular trap formation. Blood *111*, 3070-3080.

Vong, L., Lorentz, R.J., Assa, A., Glogauer, M., and Sherman, P.M. (2014). Probiotic Lactobacillus rhamnosus inhibits the formation of neutrophil extracellular traps. J Immunol *192*, 1870-1877.

Vu, A.T., Baba, T., Chen, X., Le, T.A., Kinoshita, H., Xie, Y., Kamijo, S., Hiramatsu, K., Ikeda, S., Ogawa, H.*, et al.* (2010). 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, 993 e981-983.

Vuong, C., Voyich, J.M., Fischer, E.R., Braughton, K.R., Whitney, A.R., DeLeo, F.R., and Otto, M. (2004). Polysaccharide intercellular adhesin (PIA) protects Staphylococcus epidermidis against major components of the human innate immune system. Cell Microbiol *6*, 269-275.

Wagner, T.M., Janice, J., Paganelli, F.L., Willems, R.J., Askarian, F., Pedersen, T., Top, J., de Haas, C., van Strijp, J.A., Johannessen, M.*, et al.* (2018). Enterococcus faecium TIR-Domain Genes Are Part of a Gene Cluster Which Promotes Bacterial Survival in Blood. Int J Microbiol *2018*, 1435820.

Wang, E., Qiang, X., Li, J., Zhu, S., and Wang, P. (2016). The in Vitro Immune-Modulating Properties of a Sweat Gland-Derived Antimicrobial Peptide Dermcidin. Shock *45*, 28-32.

Wang, M., Karlsson, C., Olsson, C., Adlerberth, I., Wold, A.E., Strachan, D.P., Martricardi, P.M., Aberg, N., Perkin, M.R., Tripodi, S.*, et al.* (2008). Reduced diversity in the early fecal microbiota of infants with atopic eczema. J Allergy Clin Immunol *121*, 129-134.

Wang, R., Braughton, K.R., Kretschmer, D., Bach, T.H., Queck, S.Y., Li, M., Kennedy, A.D., Dorward, D.W., Klebanoff, S.J., Peschel, A.*, et al.* (2007). Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med *13*, 1510-1514.

Wang, Y., Edelmayer, R., Wetter, J., Salte, K., Gauvin, D., Leys, L., Paulsboe, S., Su, Z., Weinberg, I., Namovic, M.*, et al.* (2019). Monocytes/Macrophages play a pathogenic role in IL-23 mediated psoriasis-like skin inflammation. Sci Rep *9*, 5310.

Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., Hayama, R., Leonelli, L., Han, H., Grigoryev, S.A.*, et al.* (2009). Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. J Cell Biol *184*, 205-213.

Wang, Z., Mascarenhas, N., Eckmann, L., Miyamoto, Y., Sun, X., Kawakami, T., and Di Nardo, A. (2017). Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes. J Allergy Clin Immunol *139*, 1205-1216 e1206.

Wanke, I., Skabytska, Y., Kraft, B., Peschel, A., Biedermann, T., and Schittek, B. (2013). Staphylococcus aureus skin colonization is promoted by barrier disruption and leads to local inflammation. Exp Dermatol *22*, 153-155.

Wanke, I., Steffen, H., Christ, C., Krismer, B., Gotz, F., Peschel, A., Schaller, M., and Schittek, B. (2011). Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. J Invest Dermatol *131*, 382-390.

Wanner, S., Schade, J., Keinhorster, D., Weller, N., George, S.E., Kull, L., Bauer, J., Grau, T., Winstel, V., Stoy, H.*, et al.* (2017). Wall teichoic acids mediate increased virulence in Staphylococcus aureus. Nat Microbiol *2*, 16257.

Waters, J.P., Pober, J.S., and Bradley, J.R. (2013). Tumour necrosis factor in infectious disease. J Pathol *230*, 132-147.

Webster, S.J., Daigneault, M., Bewley, M.A., Preston, J.A., Marriott, H.M., Walmsley, S.R., Read, R.C., Whyte, M.K., and Dockrell, D.H. (2010). Distinct cell death programs in monocytes regulate innate responses following challenge with common causes of invasive bacterial disease. J Immunol *185*, 2968-2979.

Weidenmaier, C., Goerke, C., and Wolz, C. (2012). Staphylococcus aureus determinants for nasal colonization. Trends Microbiol *20*, 243-250.

Weidinger, S., Beck, L.A., Bieber, T., Kabashima, K., and Irvine, A.D. (2018). Atopic dermatitis. Nat Rev Dis Primers *4*, 1.

Wertheim, H.F., Melles, D.C., Vos, M.C., van Leeuwen, W., van Belkum, A., Verbrugh, H.A., and Nouwen, J.L. (2005). The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis *5*, 751-762.

Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B., and Sahl, H.G. (2001). Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J Biol Chem *276*, 1772-1779.

Willey, J.M., and van der Donk, W.A. (2007). Lantibiotics: peptides of diverse structure and function. Annu Rev Microbiol *61*, 477-501.

Williams, H., Stewart, A., von Mutius, E., Cookson, W., Anderson, H.R., International Study of, A., Allergies in Childhood Phase, O., and Three Study, G. (2008). Is eczema really on the increase worldwide? J Allergy Clin Immunol *121*, 947-954 e915.

Williams, M.R., Nakatsuji, T., Sanford, J.A., Vrbanac, A.F., and Gallo, R.L. (2017). Staphylococcus aureus Induces Increased Serine Protease Activity in Keratinocytes. J Invest Dermatol *137*, 377-384.

Wolk, K., Witte, E., Wallace, E., Docke, W.D., Kunz, S., Asadullah, K., Volk, H.D., Sterry, W., and Sabat, R. (2006). IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol *36*, 1309-1323.

Wood, L.C., Elias, P.M., Calhoun, C., Tsai, J.C., Grunfeld, C., and Feingold, K.R. (1996). Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. J Invest Dermatol *106*, 397-403.

Yamasaki, K., and Gallo, R.L. (2008). Antimicrobial peptides in human skin disease. Eur J Dermatol *18*, 11-21.

Yang, J., Zhao, L., Xu, M., and Xiong, N. (2017). Establishment and function of tissue-resident innate lymphoid cells in the skin. Protein Cell *8*, 489-500.

Yao, Z., Painter, S.L., Fanslow, W.C., Ulrich, D., Macduff, B.M., Spriggs, M.K., and Armitage, R.J. (1995). Human IL-17: a novel cytokine derived from T cells. J Immunol *155*, 5483-5486.

Yipp, B.G., Petri, B., Salina, D., Jenne, C.N., Scott, B.N., Zbytnuik, L.D., Pittman, K., Asaduzzaman, M., Wu, K., Meijndert, H.C*., et al.* (2012). Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. Nat Med *18*, 1386-1393.

Yockey, L.J., Demehri, S., Turkoz, M., Turkoz, A., Ahern, P.P., Jassim, O., Manivasagam, S., Kearney, J.F., Gordon, J.I., and Kopan, R. (2013). The absence of a microbiota enhances TSLP expression in mice with defective skin barrier but does not affect the severity of their allergic inflammation. J Invest Dermatol *133*, 2714-2721.

Yokoyama, R., Itoh, S., Kamoshida, G., Takii, T., Fujii, S., Tsuji, T., and Onozaki, K. (2012). Staphylococcal superantigen-like protein 3 binds to the Toll-like receptor 2 extracellular domain and inhibits cytokine production induced by Staphylococcus aureus, cell wall component, or lipopeptides in murine macrophages. Infection and immunity *80*, 2816-2825.

Yoo, D.G., Winn, M., Pang, L., Moskowitz, S.M., Malech, H.L., Leto, T.L., and Rada, B. (2014). Release of cystic fibrosis airway inflammatory markers from Pseudomonas aeruginosa-stimulated human neutrophils involves NADPH oxidase-dependent extracellular DNA trap formation. J Immunol *192*, 4728-4738.

Yu, J., Luo, Y., Zhu, Z., Zhou, Y., Sun, L., Gao, J., Sun, J., Wang, G., Yao, X., and Li, W. (2018). A tryptophan metabolite of the skin microbiota attenuates inflammation in patients with atopic dermatitis through the aryl hydrocarbon receptor. J Allergy Clin Immunol.

Zaiou, M., Nizet, V., and Gallo, R.L. (2003). Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. J Invest Dermatol *120*, 810-816.

Zanger, P., Holzer, J., Schleucher, R., Scherbaum, H., Schittek, B., and Gabrysch, S. (2010). Severity of Staphylococcus aureus infection of the skin is associated with inducibility of human beta-defensin 3 but not human beta-defensin 2. Infect Immun *78*, 3112-3117.

Zeeuwen, P.L., Boekhorst, J., van den Bogaard, E.H., de Koning, H.D., van de Kerkhof, P.M., Saulnier, D.M., van, S., II, van Hijum, S.A., Kleerebezem, M., Schalkwijk, J*., et al.* (2012). Microbiome dynamics of human epidermis following skin barrier disruption. Genome Biol *13*, R101.

Zheng, T., Oh, M.H., Oh, S.Y., Schroeder, J.T., Glick, A.B., and Zhu, Z. (2009). Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. J Invest Dermatol *129*, 742-751.

Zipperer, A., Konnerth, M.C., Laux, C., Berscheid, A., Janek, D., Weidenmaier, C., Burian, M., Schilling, N.A., Slavetinsky, C., Marschal, M*., et al.* (2016). Human commensals producing a novel antibiotic impair pathogen colonization. Nature *535*, 511-516.

Zlotnik, A., and Yoshie, O. (2000). Chemokines: a new classification system and their role in immunity. Immunity *12*, 121-127.

Zou, J., Baghdayan, A.S., Payne, S.J., and Shankar, N. (2014). A TIR domain protein from E. faecalis attenuates MyD88-mediated signaling and NF-kappaB activation. PLoS One *9*, e112010.

Zweemer, A.J., Toraskar, J., Heitman, L.H., and AP, I.J. (2014). Bias in chemokine receptor signalling. Trends Immunol *35*, 243-252.

9 Appendix

9.1 Accepted Publication I

Burian M., Bitschar K., Dylus B., Peschel A. and Schittek B. The Protective Effect of Microbiota on *S. aureus* Skin Colonization Depends on the Integrity of the Epithelial Barrier. J Invest Dermatol. 2017, April; 137(4):976-979; doi: 10.1016/j.jid.2016.11.024.

The Protective Effect of Microbiota on *S. aureus* Skin Colonization Depends on the Integrity of the Epithelial Barrier

Journal of Investigative Dermatology (2017) **137**, 976–979; doi:10.1016/j.jid.2016.11.024

TO THE EDITOR

Human skin, as the primary interface between the body and the environment, is constantly exposed to an immense number of potential pathogens, and at the same time it allows commensal bacteria to colonize and form tissue-specific microbiota. The mechanisms responsible for selective colonization of the skin are incompletely understood but are influenced by endogenous host factors, exogenous environmental factors, and innate and adaptive immune responses (Cogen et al., 2008; Naik et al., 2015; Scharschmidt et al., 2015). Among the skin's complex ecosystem, *Staphylococci* species are considered the most important colonizers, both in terms of their beneficial role as part of the skin microbiota and as a source for infection (Cogen et al., 2008). As part of the skin microbiota *Staphylococcus epidermidis* ubiquitously colonizes human skin; in fact, it is the most frequently isolated skin commensal in humans (Grice and Segre, 2011; SanMiguel and Grice, 2015). On the other hand, *Staphylococcus aureus* is only rarely found on healthy human skin but is frequently found on the skin of atopic dermatitis patients, who additionally often suffer from disruption of the epithelial barrier (Schittek, 2011). Overabundance of cutaneous *S. aureus*, especially during atopic dermatitis flares, is associated with a loss of microbiome diversity, indicating that the skin microbiome shapes *S. aureus* skin colonization (Kong et al., 2012). It has previously been shown that *S. epidermidis* contributes to the prevention of pathogen skin colonization by cross-inhibition of the *S. aureus* accessory gene regulator system (Otto et al., 1999) by the production of a protective lipopeptide (Lai et al., 2010; Li et al., 2013) or by producing phenol-soluble modulins, which can cooperate with host-derived antimicrobial peptides to kill pathogens (Cogen et al., 2010). However, the effect of *S. epidermidis* on pathogen colonization on inflamed and non-inflamed skin has been investigated insufficiently. In our study we analyzed the influence of the skin-resident bacterium *S. epidermidis* on *S. aureus* skin colonization and invasion by in vitro and in vivo infection models using either primary human keratinocytes or an epicutaneous mouse skin infection model in intact and disrupted skin (Burian et al., 2015; Wanke et al., 2013; Zipperer et al., 2016).

In the in vitro infection model, differentiated primary human keratinocytes were pretreated with *S. epidermidis* 1457 using a multiplicity of infection of 30 for 2 hours or *S. epidermidis*-conditioned medium (CM) for 24 hours (Figure 1a). Subsequently, cells were infected (multiplicity of infection = 30) with the *S. aureus* strains USA300 (Figure 1b) or SA113 (Figure 1c) for 1.5 hours, followed by either cell lysis (adhesion and invasion) or lysostaphin treatment for 1.5 hours (invasion) and subsequent cell lysis to analyze invaded bacteria only. Serial dilutions of the lysates were spotted onto blood agar plates and colony-forming units (CFUs) were determined. The data shown in Figure 1b and c indicate that *S. epidermidis* significantly protects primary human keratinocytes against *S. aureus* adhesion and invasion. This protective effect could be observed by using either live *S. epidermidis* bacterial cells or *S. epidermidis* CM only, indicating that *S. epidermidis*-secreted factors are responsible for immune conditioning of the epithelial barrier. Similar results could be obtained by using CM of other *S. epidermidis* strains, including four skin-derived commensal strains from healthy individuals (SIHI 1–4) and two commensal strains from the skin of patients suffering from inflammatory skin diseases (CI-1, CI-2), indicating that this is a general effect of *S. epidermidis* (Figure 1d). The protection was neither observed after pretreatment with CM of *S. aureus* itself nor after pretreatment with other commensal *Staphylococcus* strains isolated from healthy skin (Figure 1e). Merely CM of *Corynebacterium pseudodiphtheriticum* induced a visible, however not significant, reduction in *S. aureus* colonization.

To examine whether the protective effect can also be observed in vivo, we used an epicutaneous skin colonization model we previously described (Burian et al., 2015; Wanke et al., 2013; Zipperer et al., 2016). Briefly, 1×10^9 live *S. epidermidis* bacterial cells, *S. epidermidis* CM, or phosphate buffered saline as a control was epicutaneously applied 24 hours before *S. aureus* USA300 application on the back skin of shaved C57BL/6 mice (Figure 2a). Skin samples were taken 24 hours after *S. aureus* application, and the numbers of surface-attached bacteria and of bacteria located in the deeper skin tissue were determined. Mice pretreated with live *S. epidermidis* bacterial cells or *S. epidermidis* CM showed significantly fewer CFUs compared with control mice (Figure 2b and c). These results indicate that *S. epidermidis* and its secreted factors induce a protective mechanism in the skin that prevents *S. aureus* from colonizing. However,

Abbreviations: CFU, colony-forming unit; CM, conditioned medium; MOI, multiplicity of infection

Accepted manuscript published online 3 December 2016; corrected proof published online 16 February 2017

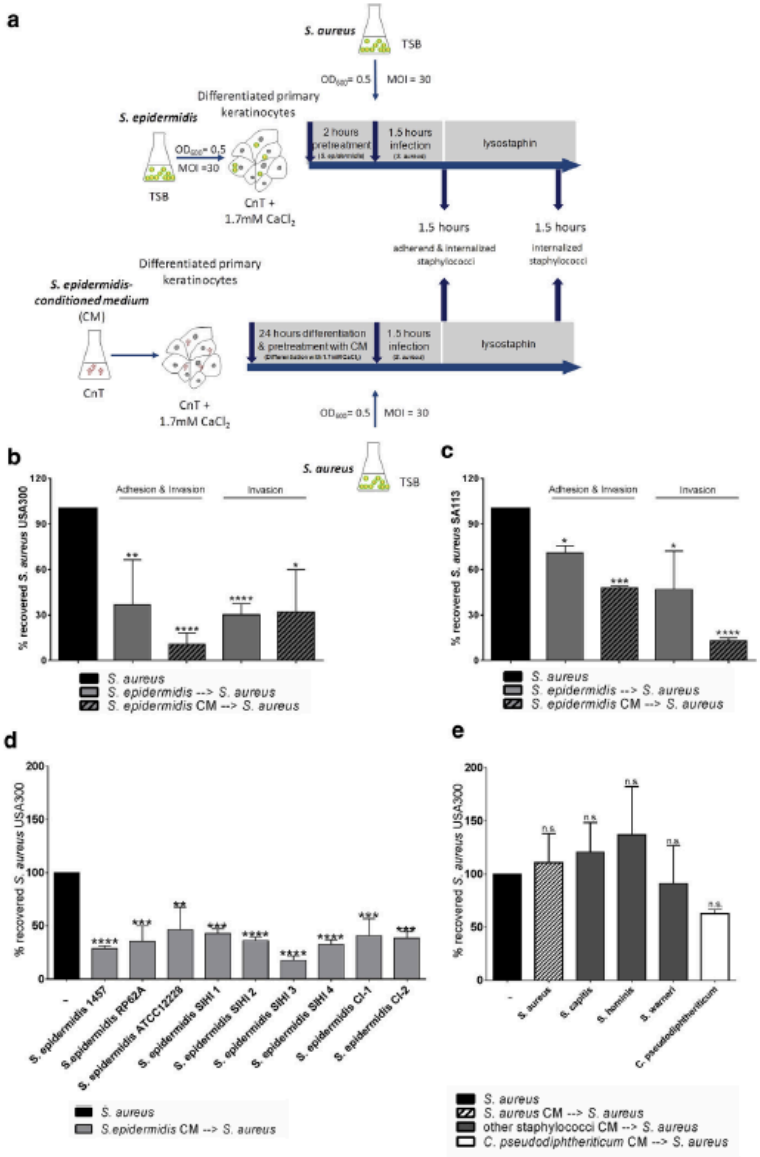


Figure 1. *S. epidermidis*-secreted factors protect primary human keratinocytes against *S. aureus* infection. (**a**) Infection model and experimental time course. Treatment of differentiated primary human keratinocytes with live *S. epidermidis* bacterial cells or *S. epidermidis*-conditioned medium before *S. aureus* infection. At 1.5 hours after *S. aureus* infection, adherent and internalized staphylococci species were analyzed, and 3 hours after initial *S. aureus* infection, only internalized staphylococci species were determined. (**b, c**) Capability of *S. aureus* to adhere to and invade primary human keratinocytes. Differentiated primary human keratinocytes were pretreated with *S. epidermidis* 1457 (MOI = 30) for 2 hours or *S. epidermidis*-conditioned mediumfor 24 hours. Subsequently, cells were infected (MOI = 30) with *S. aureus* (**b**) USA300 or (**c**) SA113 for 1.5 hours, followed either by cell lysis (adhesion and invasion) or lysostaphin-treatment for 1.5 hours (invasion). Serial dilutions of the lysate were spotted on blood agar plates and CFUs were determined. Data represent mean values ± standard error of the mean of three independent experiments. Significant differences to the untreated control were analyzed by ordinary one-way analysis of variance followed by Dunnett posttest: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (**d, e**) Capability of CM of different *S. epidermidis* isolates or of other skin commensals to protect against *S. aureus* adhesion and invasion. Differentiated primary human keratinocytes were pretreated with indicated CM for 24 hours. Subsequently, cells were infected (MOI = 30) with *S. aureus* USA300 for 1.5 hours followed by cell lysis (adhesion and invasion). Serial dilutions of the lysate were spotted on blood agar plates, and CFUs were determined. Data represent mean values ± standard error of the mean of three independent experiments. Significant differences to the untreated control were analyzed by ordinary one-way analysis of variance, followed by Dunnett posttest: $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. CFU, colony-forming unit; CM, conditioned medium; CnT, epidermal keratinocyte medium; M, mol/L; MOI, multiplicity of infection; n.s., not significant; OD, optical density; TSB, tryptic soy broth.

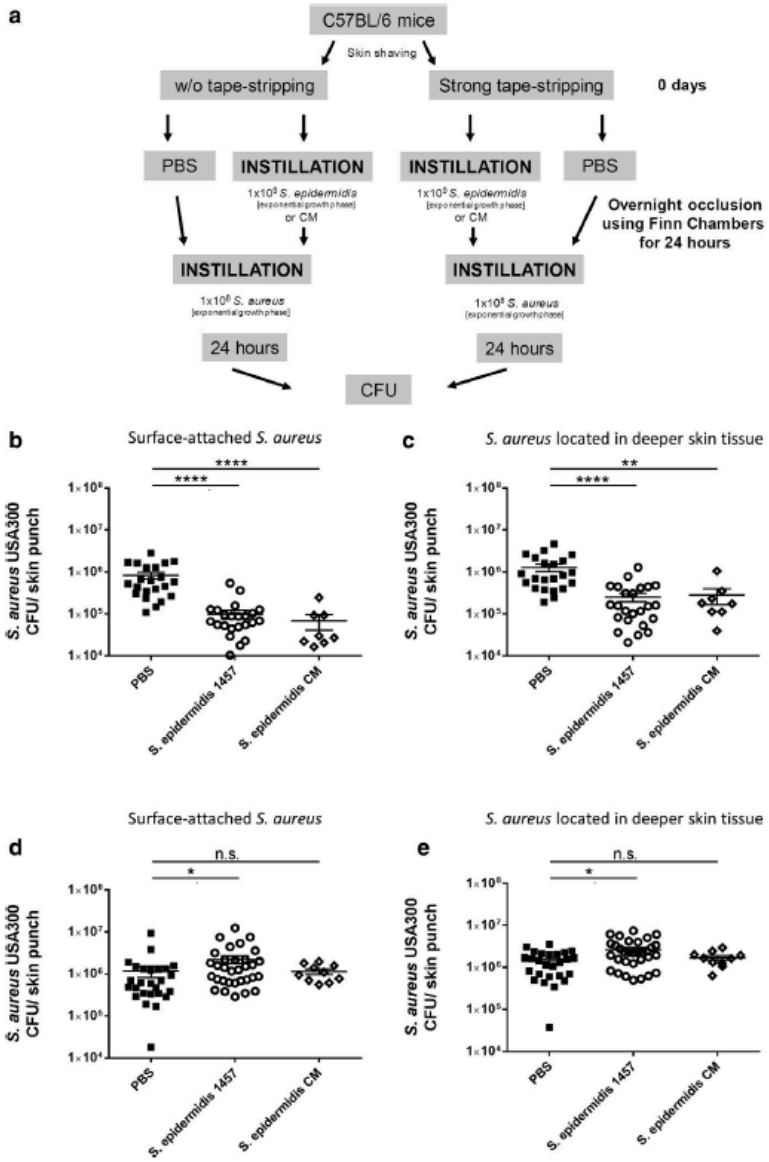


Figure 2. *S. epidermidis*-induced skin protection against *S. aureus* infection depends on the integrity of the epithelial barrier. (**a**) Epicutaneous mouse skin infection model. 1×10^9 live *S. epidermidis* bacterial cells, *S. epidermidis* CM, or PBS as a control were epicutaneously applied 24 hours before *S. aureus* USA300 application on the back skin of shaved C57BL/6 mice. Skin of mice was either unaffected or superficially disrupted by multiple stripping with an adhesive tape. (**b, c**) Mouse infection of unaffected skin. Unaffected back skin of C57BL/6 mice was pretreated with either 1×10^9 live *S. epidermidis* bacterial cells, *S. epidermidis* CM, or PBS as a control 24 hours before *S. aureus* application. After 1 day the number of (**b**) *S. aureus* CFUs for surface-attached bacteria and (**c**) bacteria located in deeper skin tissue was determined. Horizontal lines represent the mean of each group ± standard error of the mean. Significant differences to the PBS control were analyzed by ordinary one-way analysis of variance followed by Dunnett posttest: $^{**}P < 0.01$, $^{****}P < 0.0001$. (**d, e**) Mouse infection of superficially disrupted skin. Tape-stripped back skin of C57BL/6 mice was pretreated with either 1×10^9 live *S. epidermidis* bacterial cells, *S. epidermidis* CM, or PBS as a control 24 hours before *S. aureus* application. After 1 day the number of *S. aureus* CFUs for (**d**) surface-attached bacteria as well as (**e**) bacteria located in deeper skin tissue was determined. Horizontal lines represent the mean of each group ± standard error of the mean. Significant differences to the PBS control were analyzed by ordinary one-way analysis of variance followed by Dunnett posttest: $^{*}P < 0.05$. CFU, colony-forming unit; CM, conditioned medium; n.s., not significant; PBS, phosphate buffered saline.

induction of skin inflammation by multiple tape-stripping (Wanke et al., 2013) completely eradicated the *S. epidermidis*-induced protective effect by living bacteria and CM (Figure 2d and e). Under these conditions we could even observe a significant increase in *S. aureus* skin colonization after pretreatment with live bacterial cells, not when skin was pretreated with *S. epidermidis* CM, indicating that a cell-bound component of *S. epidermidis* is responsible for the enhanced *S. aureus* colonization on inflamed skin.

In summary, we show that *S. epidermidis* as a member of the skin microbiota has a protective effect on *S. aureus* skin colonization. This effect, however, depends on the integrity of the epithelial barrier and is reversed by epithelial barrier disruption, which is often associated with skin inflammation. On the basis of our previous results, with which we showed that *S. epidermidis* amplifies the innate immune response in human skin (Wanke et al., 2011), we propose that in healthy skin the microbiota creates a protective environment by immune conditioning of the epithelial surface toward a protective immune response. However, barrier disruption generates an inflammatory environment, which itself promotes pathogen colonization and infection leading to suppression of the protective mechanism of skin commensals. This adverse effect of microbiota might be a general phenomenon in several inflammatory skin diseases such as atopic dermatitis. Further studies will elucidate the signaling pathways involved in *S. epidermidis*-induced modulation of the immune response toward *S. aureus* skin infection and also the bacterial factors that trigger both the protective and the adverse effects.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was funded by the Deutsche Forschungsgemeinschaft to BS and AP (TRR156 and Schi510/8-1, PE805/5-1). The animal experiments were performed with the approval of the local authorities (Regierungspräsidium Tübingen HT1/12). The use of human skin tissue was approved by the medical ethical committee of the University of Tübingen and was performed in accordance with the Declaration of Helsinki principles (331/2010BO2).

Marc Burian[1], Katharina Bitschar[1], Beatrice Dylus[1], Andreas Peschel[2] and Birgit Schittek[1,]*

[1]*Department of Dermatology, University of Tübingen, 72076 Tübingen, Germany; and* [2]*Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, 72076 Tübingen, Germany*

[*]*Corresponding author e-mail: birgit.schittek@med.uni-tuebingen.de*

REFERENCES

Burian M, Velic A, Matic K, Gunther S, Kraft B, Gonser L, et al. Quantitative proteomics of the human skin secretome reveal a reduction in immune defense mediators in ectodermal dysplasia patients. J Invest Dermatol 2015;135:759–67.

Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? Br J Dermatol 2008;158:442–55.

Cogen AL, Yamasaki K, Muto J, Sanchez KM, Crotty Alexander L, Tanios J, et al. Staphylococcus epidermidis antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A Streptococcus. PLoS One 2010;5:e8557.

Grice EA, Segre AJ. The skin microbiome. Nat Rev Microbiol 2011;9:244–53.

Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res 2012;2:850–9.

Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A, et al. Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. J Invest Dermatol 2010;130:2211–21.

Li D, Lei H, Li Z, Li H, Wang Y, Lai Y. A novel lipopeptide from skin commensal activates TLR2/CD36-p38 MAPK signaling to increase antibacterial defense against bacterial infection. PLoS One 2013;8:e58288.

Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, et al. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. Nature 2015;520:104–8.

Otto M, Süßmuth R, Vuong C, Jung G, Götz F. Inhibition of virulence factor expression in Staphylococcus aureus by the Staphylococcus epidermidis agr pheromone and derivatives. FEBS Lett 1999;450:257–62.

SanMiguel A, Grice EA. Interactions between host factors and the skin microbiome. Cell Mol Life Sci 2015;72:1499–515.

Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, Nosbaum A, et al. A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes. Immunity 2015;43:1011–21.

Schittek B. The antimicrobial skin barrier in patients with atopic dermatitis. Curr Probl Dermatol 2011;41:54–67.

Wanke I, Skabytska Y, Kraft B, Peschel A, Biedermann T, Schittek B. Staphylococcus aureus skin colonization is promoted by barrier disruption and leads to local inflammation. Exp Dermatol 2013;22:153–5.

Wanke I, Steffen H, Christ C, Krismer B, Gotz F, Peschel A, et al. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. J Invest Dermatol 2011;131:382–90.

Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, Weidenmaier C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. Nature 2016;535:511–6.

9.2 Accepted Publication II

Bitschar K., Sauer B., Focken J., Dehmer H., Moos S., Konnerth M., Schilling N.A., Grond S., Kalbacher H., Kurschus F.C., Götz F., Krismer B., Peschel A. and Schittek B. Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. Nat Commun. 2019 Jun 21;10(1):2730. doi: 10.1038/s41467-019-10646-7.

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-10646-7 OPEN

Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors

Katharina Bitschar[1], Birgit Sauer[1], Jule Focken[1], Hanna Dehmer[1], Sonja Moos[2], Martin Konnerth[3], Nadine A. Schilling[3], Stephanie Grond[3], Hubert Kalbacher[4], Florian C. Kurschus[2,5], Friedrich Götz[6], Bernhard Krismer[7,8], Andreas Peschel[7,8] & Birgit Schittek[1]

Recently our groups discovered lugdunin, a new cyclic peptide antibiotic that inhibits *Staphylococcus aureus* epithelial colonization in humans and rodents. In this work, we analyzed its immuno-modulatory and antimicrobial potential as a single agent or in combination with other microbiota- or host-derived factors. We show that pretreatment of primary human keratinocytes or mouse skin with lugdunin in combination with microbiota-derived factors results in a significant reduction of *S. aureus* colonization. Moreover, lugdunin increases expression and release of LL-37 and CXCL8/MIP-2 in human keratinocytes and mouse skin, and results in the recruitment of monocytes and neutrophils in vivo, both by a TLR/MyD88-dependent mechanism. Interestingly, *S. aureus* elimination by lugdunin is additionally achieved by synergistic antimicrobial activity with LL-37 and dermcidin-derived peptides. In summary, our results indicate that lugdunin provides multi-level protection against *S. aureus* and may thus become a promising treatment option for *S. aureus* skin infections in the future.

[1] Department of Dermatology, University of Tübingen, Liebermeisterstraße 25, 72076 Tübingen, Germany. [2] Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Langenbeckstraße 1, 55131 Mainz, Germany. [3] Institute of Organic Chemistry, University of Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany. [4] Interfaculty Institute of Biochemistry, University of Tübingen, Ob dem Himmelreich 7, 72074 Tübingen, Germany. [5] Department of Dermatology, Heidelberg University Hospital, Im Neuenheimer Feld 440, 69120 Heidelberg, Germany. [6] Interfaculty Institute of Microbiology and Infection Medicine, Microbial Genetics, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany. [7] Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany. [8] German Centre for Infection Research (DZIF), Partner Site Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany. Correspondence and requests for materials should be addressed to B.S. (email: Birgit.schittek@med.uni-tuebingen.de)

Skin is a challenging habitat for bacteria with conditions, including dryness, low nutrient availability, high salt concentrations, and low pH, as well as the presence of host antimicrobial peptides (AMPs) and lipids[1]. Nevertheless, human skin is populated by a complex microbiota whose composition is mainly determined by the ecologic feature of the body site[2,3], but is also largely influenced by host- and bacteria-derived factors. During steady state, a constant interplay among them allows for colonization with commensal microorganisms, while at the same time pathogenic microorganisms such as *Staphylococcus aureus* can be efficiently prevented from persisting.

Characteristic changes in the composition of skin microbial consortia have been associated with chronic skin disorders such as atopic dermatitis (AD)[4]. Usually, *S. aureus* can hardly be found on healthy skin and only in 30% of the human population in the anterior nares[5], but it is abundant on inflamed and non-inflamed skin of AD patients[4]. Interestingly, overabundance of cutaneous *S. aureus*, especially during AD flares, is associated with loss of microbiome diversity, indicating that the skin microbiome shapes *S. aureus* skin colonization[3,4]. However, the mechanisms that are used by the skin microbiota during steady state to prevent colonization by *S. aureus* still remain elusive. Only recently we showed that the skin commensal *S. epidermidis* is able to amplify the innate immune response of the skin against pathogens by creating a protective environment, which ultimately leads to reduction of *S. aureus* colonization[6].

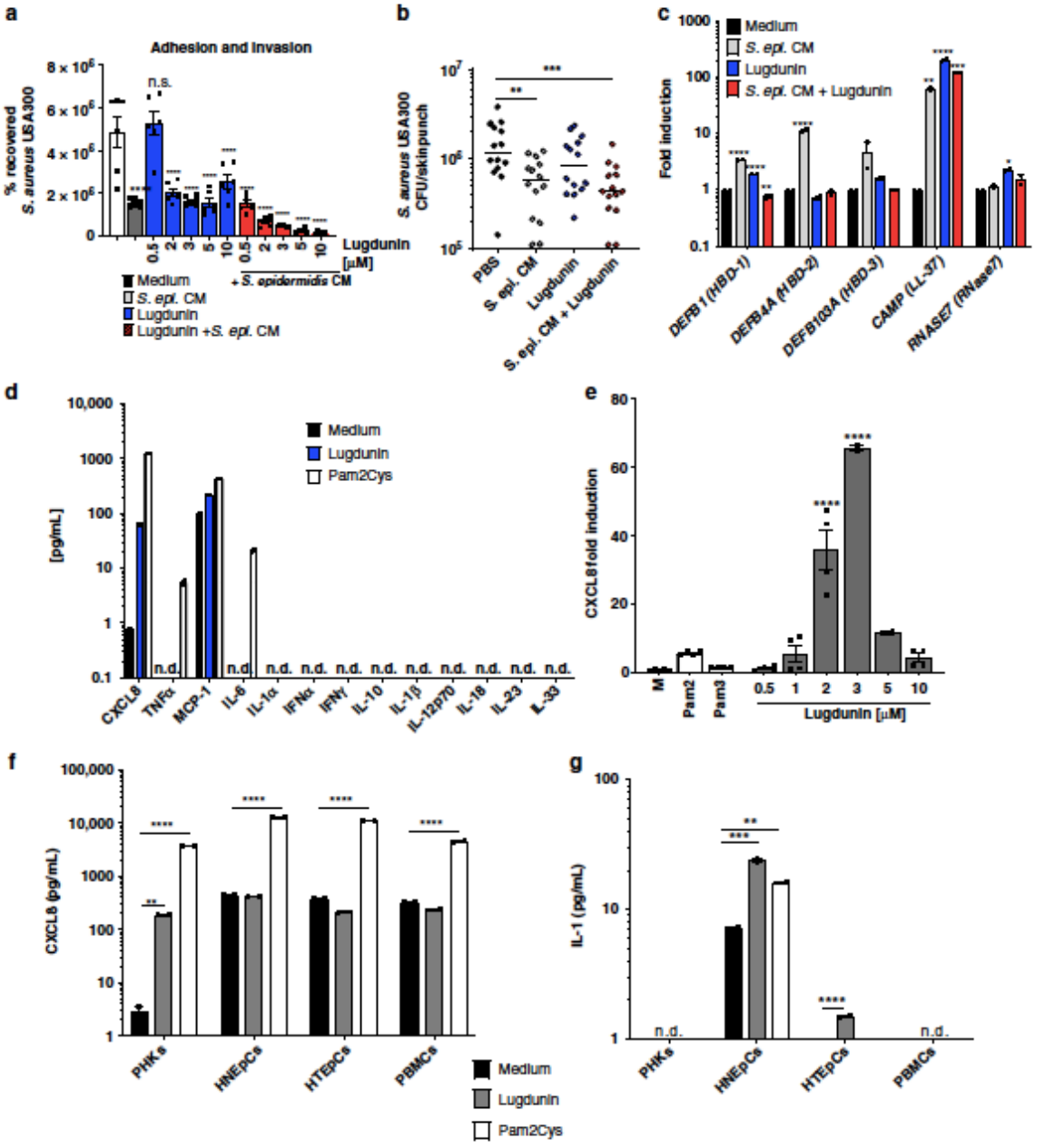
Apart from occupying space and triggering innate immune responses, the microbiota shields our skin from pathogen colonization by the release of specific AMPs called bacteriocins that can directly act on competing bacteria. Commensal-produced factors were shown to directly inhibit *S. aureus* growth[7,8]. Coagulase-negative staphylococci are frequent producers of post-translationally modified lanthionine-containing bacteriocins (lantibiotics)[9]. Recently, we discovered a novel peptide antibiotic produced by the nasal and skin commensal *Staphylococcus lugdunensis*, named lugdunin[10]. This newly discovered compound is a thiazolidine-containing cyclic peptide antibiotic, which is non-ribosomally synthesized and belongs to a new class of antibacterials[10]. Lugdunin displays potent antimicrobial activity against a wide range of Gram-positive bacteria including *S. aureus*. Importantly, humans who carry *S. lugdunensis* have a 6-fold lower risk of *S. aureus* nasal carriage[10].

We previously showed that lugdunin efficiently reduces *S. aureus* skin and nasal colonization[10]; however, the inhibitory mechanism is not completely understood. In addition to direct killing, lugdunin might reduce *S. aureus* colonization indirectly by modulation of skin defense mechanisms or by a combination of both. In fact, potential immuno-modulatory properties of bacterial AMPs such as lugdunin have rarely been investigated. In contrast, it is well established that host-derived AMPs such as the human β-defensins (HBD) 1–3 and the cathelicidin LL-37 are not only able to kill a diverse set of microorganisms but also modulate innate immune responses[11]. Here, we demonstrate that lugdunin prevents *S. aureus* colonization not only by a direct killing mechanism but also by additionally triggering increased innate defense of epithelial cells. Furthermore, synergistic and/or antagonistic activities between bacterial- and host-derived AMPs further contribute to *S. aureus* colonization resistance, which might be a common phenomenon in the complex interplay of microbes and host.

Results

Lugdunin amplifies commensal-induced *S. aureus* protection. Recently, we showed that the novel peptide antibiotic lugdunin, produced by the nasal commensal *S. lugdunensis*, effectively interferes with *S. aureus* epithelial colonization in rodents and humans[10]. On the one hand, this results from the direct bactericidal effect of lugdunin against *S. aureus*, but on the other hand, there might be an additional mechanism mediated by immune conditioning of epithelial cells by lugdunin. We proposed that lugdunin might sensitize epithelial cells towards an enhanced innate response, which prevents *S. aureus* colonization similar to the protective effect mediated by secreted factors of the skin commensal *S. epidermidis*, which was recently described by our group[6]. Therefore, we first tested whether pretreatment of primary human keratinocytes (PHKs) as well as mouse skin with lugdunin interferes with *S. aureus* colonization. As it is shown in Fig. 1a pretreatment of PHKs with lugdunin alone at concentrations of 2 µM and above significantly reduced the number of adhering *S. aureus*. Similarly, treatment of mouse skin with lugdunin alone resulted in a slight, but not significant, reduction of colonizing *S. aureus* (Fig. 1b). These reductions in *S. aureus* colonization were significantly enhanced when lugdunin was combined with *S. epidermidis* conditioned medium (CM) (Fig. 1a, b). These data indicate that lugdunin is able to sensitize keratinocytes towards a protective response against *S. aureus* skin colonization and that lugdunin and factors produced by other skin commensals act in concert.

Lugdunin induces LL-37 and CXCL8 in keratinocytes. To elucidate the mechanism of the lugdunin-induced protective response, we analyzed whether lugdunin is able to induce the expression of AMPs or pro-inflammatory cytokines in PHKs, either alone or in combination with *S. epidermidis* CM. PHKs express a basal level of the β-defensins HBD-1, HBD-2, and HBD-3, as well as LL-37 and RNase7[12]. *Staphylococcus epidermidis* CM was able to significantly induce the expression of HBD-1, HBD-2, and LL-37 (Fig. 1c), confirming our previous studies[13]. More importantly, lugdunin treatment of PHKs alone significantly induced expression and release of LL-37 in a dose-dependent manner as well as expression of HBD-1 and RNase7 (Fig. 1c and Supplementary Fig. 1). Surprisingly, combined treatment of PHKs with *S. epidermidis* CM and lugdunin abolished the effect of *S. epidermidis* CM on the induction of the respective AMPs, except for LL-37 (Fig. 1c). Next, we analyzed, whether lugdunin is able to induce the secretion of a set of 13 different pro-inflammatory cytokines and chemokines in PHKs. Interestingly, increasing lugdunin concentrations up to 3 µM specifically induced expression and release of chemokine (C-X-C motif) ligand 8 (CXCL8) in PHKs (Fig. 1d, e and Supplementary Fig. 1), while higher concentrations did not induce CXCL8 production (Fig. 1e and Supplementary Fig. 1). Conversely, expression and release of LL-37 increased with higher lugdunin concentrations (Supplementary Fig. 1). Furthermore, we topically applied lugdunin on a human 3D skin equivalent and confirmed the lugdunin-induced LL-37 and CXCL8 secretion (Supplementary Fig. 1). Additionally, expression of LL-37 in keratinocytes was confirmed by immunohistochemical stainings of mouse skin sections upon epicutaneous application of lugdunin and *S. lugdunensis* (Supplementary Fig. 1). Of note, synthetic lugdunin resulted in similar CXCL8 expression levels in PHKs as the natural lugdunin. However, the non-antimicrobial *N*-acetyl-lugdunin, on the other hand, did not induce CXCL8 expression in PHKs (Supplementary Fig. 1). Notably, lugdunin was also able to induce CXCL8 expression in primary human nasal and tracheal epithelial cells (HNEpCs and HTEpCs), as well as in peripheral blood mononuclear cells (PBMCs) (Supplementary Fig. 1), but with a much lower efficacy. Of note, basal protein levels of CXCL8 in these cells were already higher compared to PHKs and could not be further



increased by lugdunin treatment (Fig. 1f). In HNEpCs and HTEpCs, but not in PHKs and PBMCs, lugdunin was also able to induce the release of interleukin-1α (IL-1α), another important pro-inflammatory cytokine (Fig. 1d, g). Therefore, lugdunin induces different sets of pro-inflammatory cytokines in a cell-type-specific manner. Noteworthy, lugdunin treatment was not toxic to the cell types analyzed (Supplementary Fig. 1). In summary, our data indicate that lugdunin is able to induce the expression of LL-37 and pro-inflammatory cytokines in PHKs, which might modulate the response of PHKs towards *S. aureus* skin colonization.

Lugdunin-induced cytokine production is TLR/MyD88 dependent. CXCL8 expression can be induced in PHKs and other cell types by activation of the Toll-like receptor 2 (TLR2) signaling pathway[14]. Indeed, as shown in Fig. 1d, stimulation with the TLR2 ligand Pam2Cys led to a strong induction of CXCL8 secretion in PHKs, roughly 10-fold higher than lugdunin-induced CXCL8 secretion. Lugdunin is a thiazolidine-containing cyclic peptide and as such has not been described to activate TLR2 signaling. Therefore, we analyzed the potential role of TLR2 in lugdunin-induced CXCL8 release. Since PHKs constitutively express TLR2[15], we used HEK293 cells, which do not express

Fig. 1 Lugdunin sensitizes epithelial cells for innate immune defense. **a** Primary human keratinocytes (PHKs) were pretreated with *S. epidermidis* conditioned medium (CM), indicated lugdunin concentrations, or the combination of both for 20 h. Subsequently, cells were infected with *S. aureus* for 1.5 h followed by cell lysis and determination of colony-forming units (CFUs). Shown is one representative experiment of three independent experiments with six technical replicates ± s.e.m. n.s., not significant. **b** Dorsal skin of mice was pretreated with *S. epidermidis* CM, 1.5 µg lugdunin alone or in combination with *S. epidermidis* CM for 24 h. Subsequently, *S. aureus* CFUs were determined. Horizontal lines represent the mean of each group ± s.e.m. **c** PHKs were treated with 2 µM lugdunin or with *S. epidermidis* CM alone or in combination for 20 h and subsequently expression of indicated antimicrobial peptides (AMPs) (respective protein names in brackets) was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. **d** PHKs were treated with 2 µM lugdunin or 100 ng/mL Pam2Cys for 5 h and subsequently the concentration of indicated cytokines in the supernatant was analyzed by LEGENDplex™ (BioLegend). Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. **e** PHKs were treated with increasing concentrations of lugdunin or 100 ng/mL Pam2Cys or Pam3Cys for 5 h and subsequently expression of chemokine (C-X-C motif) ligand 8 (CXCL8) was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments, each with two technical replicates ± s.e.m. **f**, **g** Indicated cells were treated with 2 µM lugdunin or 100 ng/mL Pam2Cys as a positive control for 5 h and subsequently the concentration of CXCL8 (**f**) and interleukin-1α (IL-1α) (**g**) in the supernatant was analyzed by LEGENDplex™ (BioLegend) and enzyme-linked immunosorbent assay (ELISA) (R&D Systems). Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. Significant differences to control treatments were analyzed by ordinary one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). n.d. = not detected. Source data are provided as a Source Data file

TLR2, and HEK293-TLR2 cells, which were transfected with a TLR2-containing plasmid leading to surface expression of TLR2[16]. We treated both cell types with lugdunin as well as with the TLR2 ligands Pam2Cys and Pam3Cys as positive controls and analyzed expression along with protein levels of CXCL8. Pam2-Cys and Pam3Cys treatment of HEK cells induced CXCL8 expression and release in a TLR2-dependent way (Supplementary Fig. 2). Interestingly, lugdunin-induced CXCL8 expression and protein release in HEK cells was also TLR2 dependent (Fig. 2a, b) in a concentration-dependent fashion (Fig. 2b). Induction levels were, however, 1000-fold lower compared to Pam2Cys/Pam3Cys-mediated TLR2 activation (Fig. 2a and Supplementary Fig. 2).

To further investigate the involvement of TLRs, we analyzed whether lugdunin is able to induce a set of cytokines in mouse skin similar to PHKs. Therefore, we epicutaneously treated C57BL/6 mouse skin with lugdunin or phosphate-buffered saline (PBS) as a control for 24 h and determined the levels of pro-inflammatory cytokines in the skin (Fig. 2c–e and Supplementary Fig. 2). Interestingly, we found that specifically IL-1α and macrophage inflammatory protein-2 (MIP-2), the functional mouse homolog of human CXCL8[17], were induced in mouse skin by lugdunin treatment (Fig. 2d, e). Levels of other cytokines such as monocyte chemotactic protein-1, granulocyte–macrophage colony-stimulating factor, tumor necrosis factor-α, IL-6, and interferon-γ did not show significant differences compared to the control group, except for the anti-inflammatory cytokine IL-10 (Supplementary Fig. 2). To further confirm the production of MIP-2 in mouse skin, we performed immunohistochemical analyses of mouse skin sections upon epicutaneous treatment with lugdunin or *S. lugdunensis*. Similarly to LL-37 (Supplementary Fig. 1), we could confirm MIP-2 production by keratinocytes in the epidermis and the hair follicles, which also correlates with the main locations for *S. lugdunensis* colonization in vivo (Fig. 2f and Supplementary Fig. 2). To analyze the importance of TLR and MyD88 signaling in lugdunin-mediated cytokine induction, we additionally analyzed cytokine levels upon lugdunin treatment in the skin of mice deficient for MyD88 (MyD88-knockout (ko)) or for TLR2, TLR3, TLR4, TLR7, and TLR9 (5xTLR-ko). Surprisingly, expression of most cytokines was not significantly different upon lugdunin treatment compared to wild-type (WT) control mice (Supplementary Fig. 2). Lugdunin was still able to induce IL-1α in mouse skin lacking MyD88 or TLRs (5xTLR-ko) (Fig. 2d). However, lugdunin-dependent induction of MIP-2 was completely impaired in mice lacking MyD88 and reduced in 5xTLR-ko mice (Fig. 2e). In summary, these data indicate that lugdunin induces CXCL8/MIP-2 in keratinocytes by a TLR/MyD88-dependent mechanism.

Epicutaneous lugdunin recruits phagocytic cells. Induction of CXCL8/MIP-2 expression in keratinocytes is an immediate and early pro-inflammatory response resulting in the recruitment of phagocytic immune cells to clear infections[18–20]. Therefore, we analyzed the composition as well as the potential recruitment of immune cells into the skin of C57BL/6 WT, MyD88-ko, and 5xTLR-ko mice 24 h after epicutaneous treatment with lugdunin or PBS (Fig. 3a–c and Supplementary Fig. 3). In line with the lugdunin-induced MIP-2 and IL-1α induction, we found significantly enhanced recruitment of monocytes and neutrophils in the skin of mice treated with lugdunin, which was completely impaired in MyD88-ko and 5xTLR-ko mice (Fig. 3d). Percentages of live $CD45^+$ cells (Fig. 3b), B, T, and NK cells, as well as total $CD11b^+$ cells and dendritic cells, were not substantially different compared to the PBS control treatment (Fig. 3c and Supplementary Fig 3). Interestingly, macrophage levels were slightly reduced in percentage upon lugdunin treatment (Fig. 3c and Supplementary Fig. 3). Additionally, we performed immunohistochemical stainings of myeloperoxidase (MPO) in mouse skin sections demonstrating that epicutaneous application of both lugdunin and the lugdunin-producing *S. lugdunensis* results in recruitment of MPO-positive cells into the dermis (Fig. 3e). Taken together, these results indicate that CXCL8/MIP-2 induction in mouse skin and in PHKs is mediated by a TLR/MyD88-dependent pathway in keratinocytes, which leads to the recruitment of phagocytic innate immune cells such as monocytes and neutrophils.

Lugdunin amplifies innate immune responses of keratinocytes. Since we showed that lugdunin is a very potent inducer of CXCL8 and AMPs in PHKs, we asked whether other bacteria- and skin-derived AMPs are equally well able to induce CXCL8 expression in PHKs. We tested the bacteriocins nisin and gallidermin, as well as pro-gallidermin, the non-bactericidal pro-form of gallidermin, and the human AMPs LL-37 and the dermcidin-derived peptides DCD-1 and DCD-1L, both of which are secreted by eccrine sweat glands and are thus constitutively present on human skin[21,22] (Table 1). As shown in Fig. 4a, compared to the other AMPs and bacteriocins, lugdunin was especially potent in inducing CXCL8 expression in PHKs leading to over 40-fold induction. Only DCD-1 treatment resulted in a 10-fold induction of CXCL8

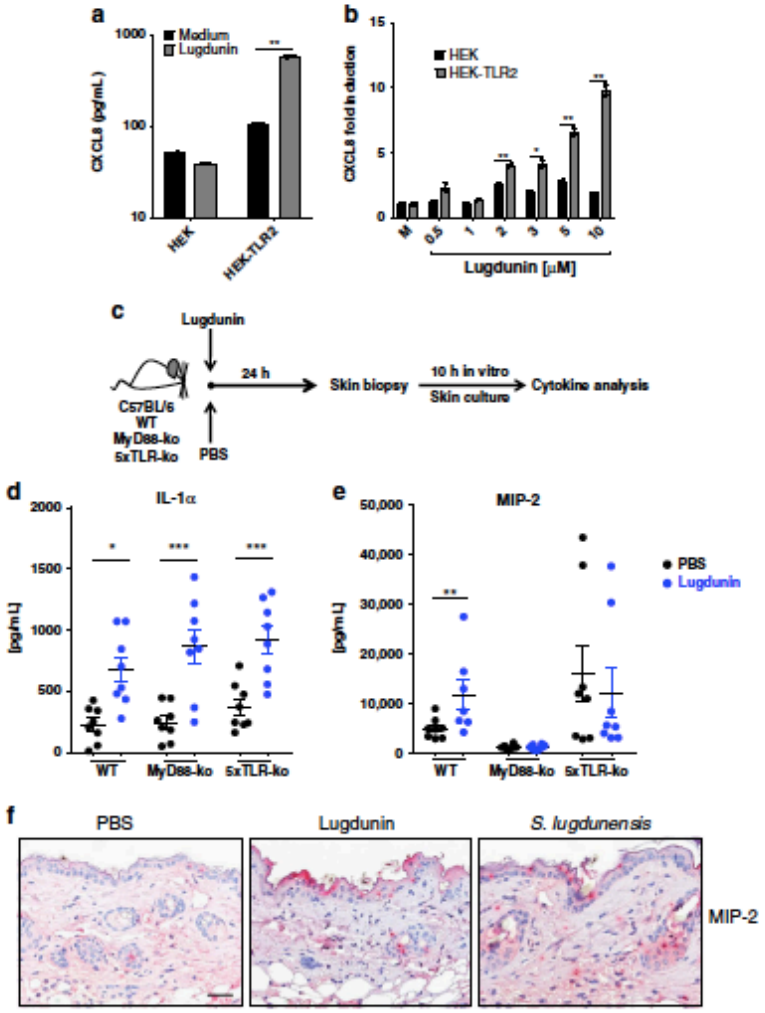


Fig. 2 Induction of chemokine (C-X-C motif) ligand 8/macrophage inflammatory protein-2 (CXCL8/MIP-2) by lugdunin is TLR2/MyD88-dependent. **a** HEK-control or HEK-TLR2 cells were treated with 2 μM lugdunin for 5 h and subsequently the CXCL8 concentration in the supernatant was analyzed by LEGENDplex™ (BioLegend). Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. **b** HEK-control or HEK-TLR2 cells were treated with increasing concentrations of lugdunin for 5 h and subsequently expression of CXCL8 was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. **c** Schematic overview of the mouse experiments: 6–8-week-old female C5BL/6 WT, MyD88-knockout (ko), or 5xTLR-ko mice were epicutaneously treated with 1.5 μg lugdunin or phosphate-buffered saline (PBS) as a control. After 24 h, mice were euthanized, 4 mm skin punches were taken, and further cultured in vitro for 10 h followed by cytokine analysis of the culture supernatant by LEGENDplex™ (BioLegend). **d**, **e** Shown are the mean concentrations of IL-1α (**d**) or MIP-2 (**e**) in the skin culture supernatant of two skin punches from four mice each ± s.e.m. Significant differences to control treatments were analyzed by an unpaired two-tailed *t* test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). **f** Representative MIP-2-stained paraffin-embedded mouse skin sections. Scale bar, 100 μM. Source data are provided as a Source Data file

expression in PHKs. LL-37, nisin, and (pro)-gallidermin, however, were not able to induce CXCL8 expression in PHKs. *Staphylococcus epidermidis* CM was equally well able to induce CXCL8 expression in PHKs as lugdunin. Interestingly, CXCL8 induction by *S. epidermidis* CM could be highly amplified by the addition of lugdunin from 40-fold to over 1000-fold (Fig. 4a). This effect was specific for lugdunin since all other AMPs/bacteriocins, except for nisin, which led to a non-significant increase in CXCL8 induction, did not amplify *S. epidermidis*-induced upregulation of CXCL8 expression in PHKs. Surprisingly, gallidermin completely blocked *S. epidermidis* CM-induced CXCL8 expression in PHKs (Fig. 4a).

Since lugdunin induced the expression of host-derived AMPs, especially LL-37 in PHKs (Fig. 1c), we asked whether combinations of lugdunin or the other bacterial antimicrobials with LL-37 or the dermcidin-derived peptides DCD-1L and DCD-1 are able to amplify CXCL8 expression in PHKs. Pam2Cys, IL-1α, or *S. epidermidis* CM treatment served as controls. None of the tested peptides exerted cytotoxicity on host cells (Supplementary Fig. 4). As shown in Fig. 4b, lugdunin treatment alone was already very

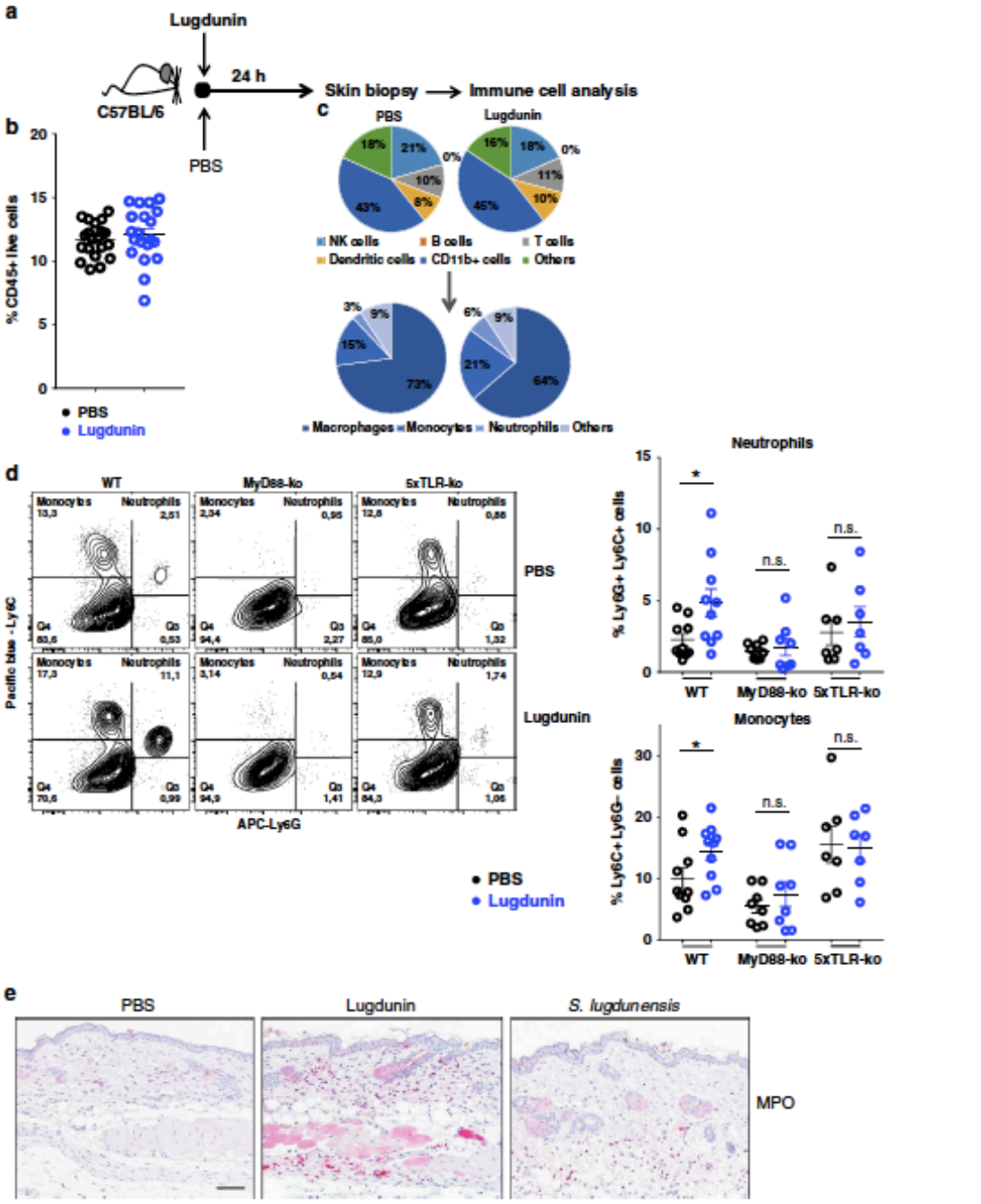


Fig. 3 Epicutaneous lugdunin recruits phagocytic cells. **a** Schematic overview of the mouse experiments: 6–8-week-old female C5BL/6 wild-type (WT), MyD88-ko, or 5xTLR-ko mice were epicutaneously treated with 1.5 µg lugdunin or phosphate-buffered saline (PBS) as a control. After 24 h, mice were euthanized, immune cells were isolated from treated skin areas, and immune cell composition was analyzed by flow cytometry. **b** Shown is the mean percentage of CD45+ live cells in mouse skin of 10 C57BL/6 WT mice ± s.e.m. One mouse is represented as two dots analyzed by two different stainings. **c** Pie charts show the mean percentage of the different immune cell subsets in the skin of 10 WT mice after 24 h of PBS or lugdunin treatment. **d** Shown are representative flow cytometry data (left panel) and the mean percentage of neutrophils (Ly6C+Ly6G+) and monocytes (Ly6C+Ly6G−) pregated on CD11b+CD45+ live cells (see Supplementary Fig. 3a, f for the gating strategy) in mouse skin ± s.e.m. One dot represents one mouse. *$P < 0.05$. **e** Representative myeloperoxidase (MPO)-stained paraffin-embedded mouse skin sections. Scale bar, 100 µM. Source data are provided as a Source Data file

Table 1 Overview of bacteriocins and human AMPs used in this study

Peptide	Sequence	Source	Mode of action
Lugdunin	CvWIVvV (cyclic thiazolidine)	*Staphylococcus lugdunensis*	Unknown
Gallidermin	IASKFLCTPGCAKTGSFNSYCC	*Staphylococcus gallinarum*	Inhibition of cell wall biosynthesis
Pro-gallidermin	MEAVKEKNELFDLDVKVNAKESNDSGAEPRIASKFLCTPGCAKTGSFNSYCC	*Staphylococcus gallinarum*	Inactive pro-form
Nisin	ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK	*Lactococcus lactis*	Pore formation, inhibition of cell wall biosynthesis
DCD-1	SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSV	Eccrine sweat glands	Pore formation
DCD-1L	SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSVL	Eccrine sweat glands	Pore formation
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	Epithelial cells, leukocytes	Pore formation

AMPs, antimicrobial peptides

potent in inducing CXCL8 expression in PHKs and the induction level was not significantly amplified further by combined treatments (Fig. 4b). These data indicate that lugdunin is a very potent immune modulator of the skin that acts in concert with other microbiota-derived modulating factors.

Lugdunin acts synergistically with host-derived AMPs. Finally, we analyzed the direct bactericidal effect of lugdunin and the bacteriocins gallidermin and nisin against *S. aureus* and asked whether lugdunin exerts antimicrobial activity in synergy with host-derived AMPs such as DCD-1L, DCD-1, and LL-37. First, we determined sub-bactericidal concentrations of the bacteriocins and AMPs listed in Table 1 against *S. aureus* USA300. The results are shown in Supplementary Fig. 5. We tested combinations of sub-bactericidal concentrations of those bacteriocins/AMPs and analyzed the activity of single or combined treatments of the methicillin-resistant *S. aureus* (MRSA) strain USA300 with these peptides. As shown in Fig. 5a–c and Supplementary Fig. 6, combinations of the human AMPs DCD-1(L) and LL-37 with sub-bactericidal concentrations of lugdunin or gallidermin and nisin resulted in enhanced *S. aureus* killing compared to the single treatments. Of note, the effect was specific for the active form of gallidermin since co-incubation with pro-gallidermin did not lead to antimicrobial activity (Supplementary Figs. 5, 6). Using CompuSyn software, we analyzed potential synergistic effects of peptide combinations and calculated the combination indices for the indicated combinations. The activities of lugdunin and gallidermin in combination with the human AMPs reached combination index (CI) values below 1 indicating synergistic activity of these peptides (Fig. 5b). To analyze whether the synergistic activity is a specific effect on *S. aureus*, we tested the spectrum of antimicrobial activity of lugdunin alone or in combination with DCD-1(L). Supplementary Figure 7 shows that the combined effect of lugdunin with DCD-1(L) could neither be observed for the Gram-positive skin commensal *S. epidermidis* or intestinal *Enterococcus faecalis* nor for the Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, or *Proteus mirabilis*. Of note, *Bacillus subtilis* was the only bacterial species tested besides *S. aureus* that was also susceptible to lugdunin and lugdunin/DCD-1(L) combinations (Supplementary Fig. 7). Additionally, we investigated whether the synergistic activity relies on the combined action of the peptides or whether one peptide is able to sensitize for bacterial killing by the other peptide. Therefore, we performed sequential incubation steps of the single peptides (Fig. 6). Single as well as combination treatments were always included as controls. Interestingly, sequential treatment of *S. aureus* with the synergistically active peptide concentrations did not lead to *S. aureus* killing (Fig. 6). From these data, we conclude that there is a synergistic activity of host- and bacteria-derived peptides in *S. aureus* clearance and that this synergistic effect seems to be dependent on a simultaneous action of the bioactive peptides.

Discussion

Lugdunin was recently discovered by our groups as a novel cyclic peptide antibiotic produced by *S. lugdunensis* that inhibits *S. aureus* nasal and skin colonization in humans and rodent models[10]. In this work, we show for the first time that lugdunin has both immuno-modulatory and bactericidal activities, both of which can be amplified by the presence of other microbiota- or host-derived factors (Fig. 7). Lugdunin particularly induced the expression of the AMP LL-37 and the pro-inflammatory chemokines CXCL8/ MIP-2 in human keratinocytes and mouse skin by a TLR/MyD88-dependent mechanism, which ultimately resulted in the recruitment of neutrophils and monocytes. Furthermore, we show that lugdunin has a synergistic antimicrobial activity against *S. aureus* in combination with skin-derived AMPs. Our results indicate that lugdunin is a multi-functional peptide protecting the host by its direct anti-bacterial activities as well as by sensitizing epithelial cells for increased defense resulting in efficient protection against *S. aureus* skin colonization.

Staphylococcus lugdunensis is most frequently found in humans in the inguinal and perineal areas, the axilla, as well as in the nail bed and the nose[10,23], where it is considered a part of the normal human skin flora. *Staphylococcus lugdunensis* can co-exist with other commensals on human skin such as *S. epidermidis*[23,24], but intriguingly, nasal colonization by *S. aureus* or *S. lugdunensis*[10,23] was shown to be mutually exclusive as a result of lugdunin production[10]. In fact, humans who are colonized by *S. lugdunensis* in the nose have a 6-fold lower risk of *S. aureus* carriage than individuals who are not colonized[10]. However, the total number of *S. lugdunensis* in the noses of carriers is considerably low compared to the number of other commensals[10]. Therefore, we speculated that apart from its antimicrobial activity, lugdunin might exhibit additional properties that contribute to the prevention of *S. aureus* colonization. Here we show that the protective potential of lugdunin can be further increased both by combined action with factors produced by other commensals that promote the host innate defense by inducing the expression of AMPs and by recruiting phagocytic immune cells, as well as by synergistic antimicrobial action with the host AMPs LL-37 and dermcidin-derived peptides. These results may explain why such

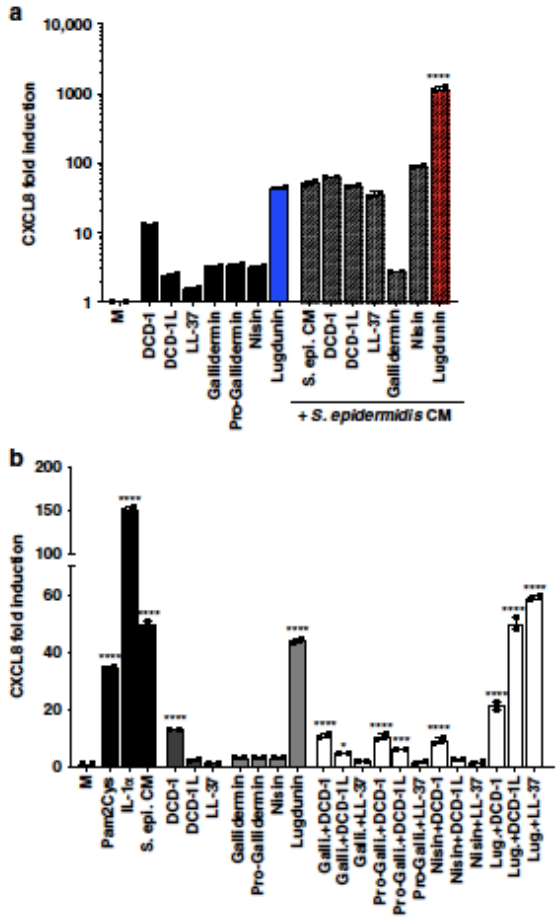


Fig. 4 Lugdunin amplifies the commensal-induced chemokine (C-X-C motif) ligand (CXCL8) induction. **a** Primary human keratinocytes (PHKs) were either treated with 2 µM of human antimicrobial peptides (AMPs) (black bars) or lugdunin (blue bars), or 0.8 µM (pro)-galliderm in or nisin (black bars), or in combination with *S. epidermidis* conditioned medium (CM) (gray and red striped bars) for 5 h, and subsequently expression of CXCL8 was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. **b** PHKs were treated with 2 µM human AMPs, 2 µM lugdunin, 0.8 µM of the other bacteriocins (gray bars) or the correspondent peptide combinations (white bars), or 50 ng/mL Pam2Cys, 10 ng/mL IL-1α, or *S. epidermidis* CM as controls (black bars), for 5 h and subsequently expression of CXCL8 was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments with two technical replicates ± s.e.m. Significant differences to control treatments were analyzed by ordinary one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (***$P < 0.001$; ****$P < 0.0001$). Source data are provided as a Source Data file

low numbers of *S. lugdunensis* can completely prevent *S. aureus* from colonizing epithelial tissues.

Bacteria from the human microbiota have been found to produce bacteriocins acting against closely related bacteria[25,26]. Lugdunin represents the founding member of the new class of cyclic thiazolidine-containing peptide antibiotics[10]. It exhibits high antimicrobial activity in the micromolar range against a wide range of Gram-positive bacteria, including MRSA, vancomycin-resistant *Enterococcus* isolates, and *Bacillus subtilis* demonstrating its high potency. In our previous work, we have shown that lugdunin treatment led to a strong reduction and even complete eradication of viable *S. aureus* on the surface and in the deeper layers of the skin of mice, demonstrating that lugdunin effectively eradicates *S. aureus* and penetrates tissues in vivo. While lugdunin did neither cause lysis of primary human neutrophils, erythrocytes, or of the human monocytic cell line HL60[10] nor displayed cell cytotoxicity on PHKs, HNEpCs, HTEpCs, or PBMCS, bacterial cells exposed to lugdunin stopped incorporating radioactive DNA, RNA, protein, or cell wall precursors even at concentrations below the minimal inhibitory concentration, suggesting that lugdunin may lead to a rapid breakdown of bacterial energy resources[10]. Thus, lugdunin can potentially act in concert with other antimicrobial substances to increase bacterial killing. In fact, here we show that lugdunin can enhance the bactericidal activity of host-derived AMPs such as LL-37 or the dermcidin-derived peptides DCD-1(L). While expression of the former can be increased by lugdunin, the latter is constantly present on human skin. Therefore, we suggest that the presence of *S. lugdunensis* on defined skin areas not only increases LL-37 expression in skin but also acts in concert with constitutively expressed host-derived AMPs and by this prevents *S. aureus* skin colonization. Moreover, the synergistic antimicrobial activity of lugdunin with host-derived AMPs seems to be mediated by a combined action since sequential incubation of *S. aureus* with these peptides has no bactericidal effect. We could speculate that lugdunin may act in a similar way as the phenol soluble modulins (PSMs) produced by *S. epidermidis*, which can bind to host-derived AMPs such as LL-37, HBD-2, and HBD-3, and thereby act in a cooperative way to kill *S. aureus*[27]. So far, it is completely unknown whether other peptide antibiotics are equally well able to induce the expression of AMPs in skin. Such synergies of host peptides with peptides derived from skin commensals have a great clinical potential: The group of Richard Gallo recently demonstrated that re-establishment of specific bacteriocin-producing skin commensals in AD patients lacking these commensals prevents *S. aureus* from colonizing the skin of these patients. In this work, they identified novel skin commensal-derived bacteriocins that act synergistically with LL-37. Additionally, a single application of these bacteriocin-producing strains significantly reduced *S. aureus* loads on the forearms of AD patients already 24 h after application[8].

It has to be determined how lugdunin production is regulated and whether factors from the host side are able to increase lugdunin production by *S. lugdunensis*. This will become especially important when considering the fact that commensals rarely express bacteriocins[10,28], but expression is induced under habitat-specific stress conditions[28,29]. Thus, we assume that staphylococci as commensals express a basal level of bacteriocins dependent on the habitat and upon entry of a pathogen to the microbial community, bacteriocin expression is further induced resulting in effective host defense.

Besides their bactericidal activity, host-derived AMPs have been shown to play a role in modulation of the innate immune defense[30] and their expression was shown to be dysregulated in AD patients[30–34]. Dermcidin and its proteolytically processed antimicrobially active peptides DCD-1(L), on the other hand, are constitutively expressed in eccrine sweat glands where they can be transported to the skin surface and serve as a constant antimicrobial shield[21,22]. Thus, being constantly present on the skin surface, our results suggest that DCD-1(L) is able to increase the innate immune defense on skin depending on the available commensal or pathogen-derived bacteriocins. Furthermore, we found that DCD-1(L) alone or in combination with bacteriocins induced CXCL8 expression in keratinocytes. These results are in

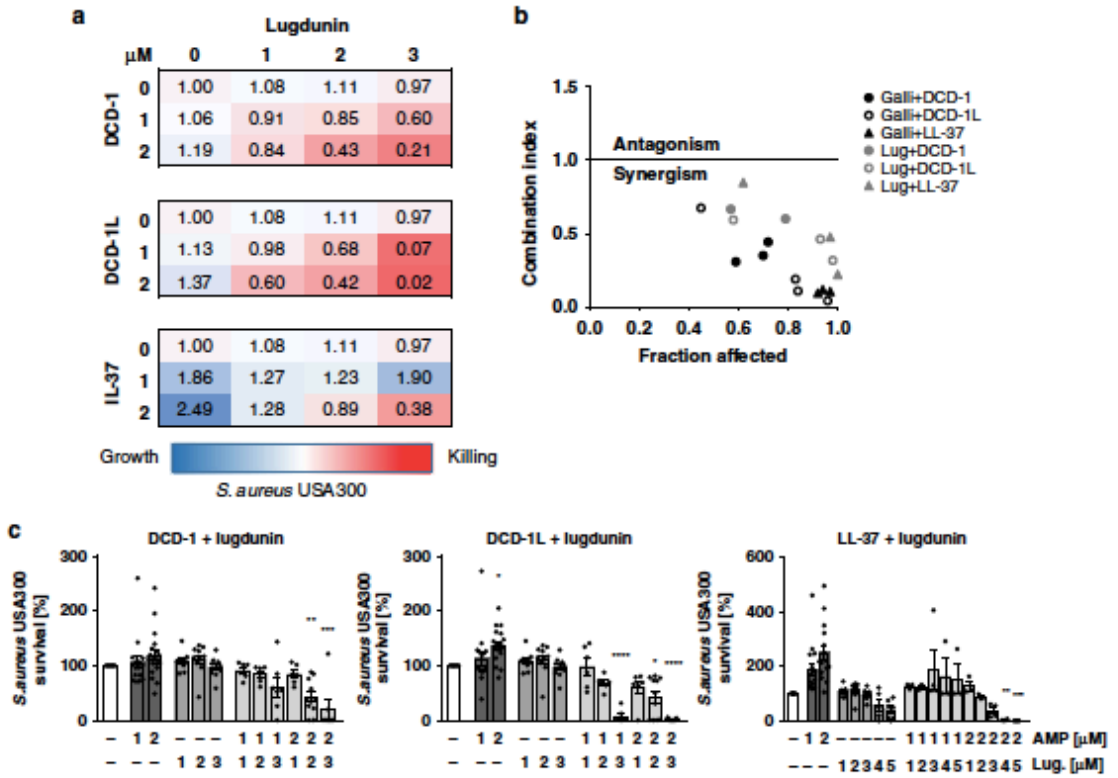


Fig. 5 Synergistic action of lugdunin and human antimicrobial peptides (AMPs) kills methicillin-resistant *S. aureus* (MRSA). **a**, **c** Logarithmically grown (3×10^6) *S. aureus* were incubated with indicated combinations of lugdunin and human AMPs in phosphate-buffered saline (PBS) containing 0.1% tryptic soy broth (TSB) at 37 °C orbital shaking (white bar, untreated; dark gray bars, AMP treatment; middle gray bars, lugdunin treatment; light gray bars, AMP and lugdunin combination treatment). After 3 h of incubation, several dilutions of the bacterial suspensions were plated onto TSB agar plates and incubated overnight at 37 °C. The next day *S. aureus* colony-forming units (CFUs) were counted. Each experiment was performed in triplicates. Data represent the percentage of CFU of at least three independent experiments normalized to the untreated control ± s.e.m. Significant differences to control treatments were analyzed by ordinary one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). **b** Combination indices (CIs) were calculated using CompuSyn (ComboSyn Inc.) and indicated in median effect plots as a function of the bacteria fractions affected by the combinatorial peptide treatment. CI values of 1 indicate additive effects, whereas values <1 and >1 indicate synergistic and antagonistic effects, respectively. Source data are provided as a Source Data file

line with Niyonsaba et al.[35] who showed that DCD-1L activates nuclear factor-κB (NF-κB) signaling in human keratinocytes and leads to the release of TNFα, CXCL8, interferon-inducible protein 10 (CXCL10), and macrophage inflammatory protein-3α (CCL20)[35]. Additionally, it was recently shown that dermcidin treatment of PBMCs induces the release of TNFα[36].

By contrast, another study showed that gallidermin was able to totally abolish staphylococcal-induced release of CXCL8 and IL-6 in dermal fibroblasts[37]. Strikingly, these data are in line with our results where gallidermin was able to suppress *S. epidermidis*-induced CXCL8 induction in PHKs. The mechanism still has to be elucidated, but it already demonstrates the potential of defined bacteriocins to counteract harmful inflammatory responses.

Keratinocytes express several pattern recognition receptors such as TLR2, which recognizes *S. aureus* lipopeptides[38]. Activation of TLR2 leads to MyD88-dependent activation of NF-κB and other transcription factors, which subsequently induce the transcription of pro-inflammatory chemokines and cytokines, such as CXCL8 and IL-1α, as well as AMPs involved in cutaneous host defense against *S. aureus*[38]. CXCL8 is a chemokine that recruits neutrophils to the site of infection, whereas IL-1α was shown to be induced in the skin by commensals where it substantially contributes to skin immunity[39,40]. Our novel finding that lugdunin, but not gallidermin or nisin, can induce the secretion of CXCL8 in PHKs and IL-1α in HNEpCs as well as MIP-2 and IL-1α in mouse skin made us speculate that lugdunin has fascinating novel properties for a bacterial peptide and can modulate host cells in a yet mysterious way. More importantly, we could find that lugdunin increases CXCL8 expression in PHKs even further in combination with other commensal-derived factors from *S. epidermidis*.

By analyzing the mechanism of lugdunin-induced CXCL8/MIP-2 induction in skin, we found that lugdunin induces CXCL8/MIP-2 by a TLR/MyD88-dependent mechanism. In MyD88-ko and 5xTLR-ko mice, MIP-2 production induced by lugdunin is impaired in contrast to IL-1α production, which is not affected. TLR2 might play a dominant role since in TLR2-expressing HEK cells lugdunin treatment results in CXCL8 expression and release. Induction levels were, however, substantially lower compared to Pam2Cys/Pam3Cys-mediated TLR2 activation, suggesting that lugdunin might be a weak TLR2 agonist or it might induce CXCL8 expression by an indirect

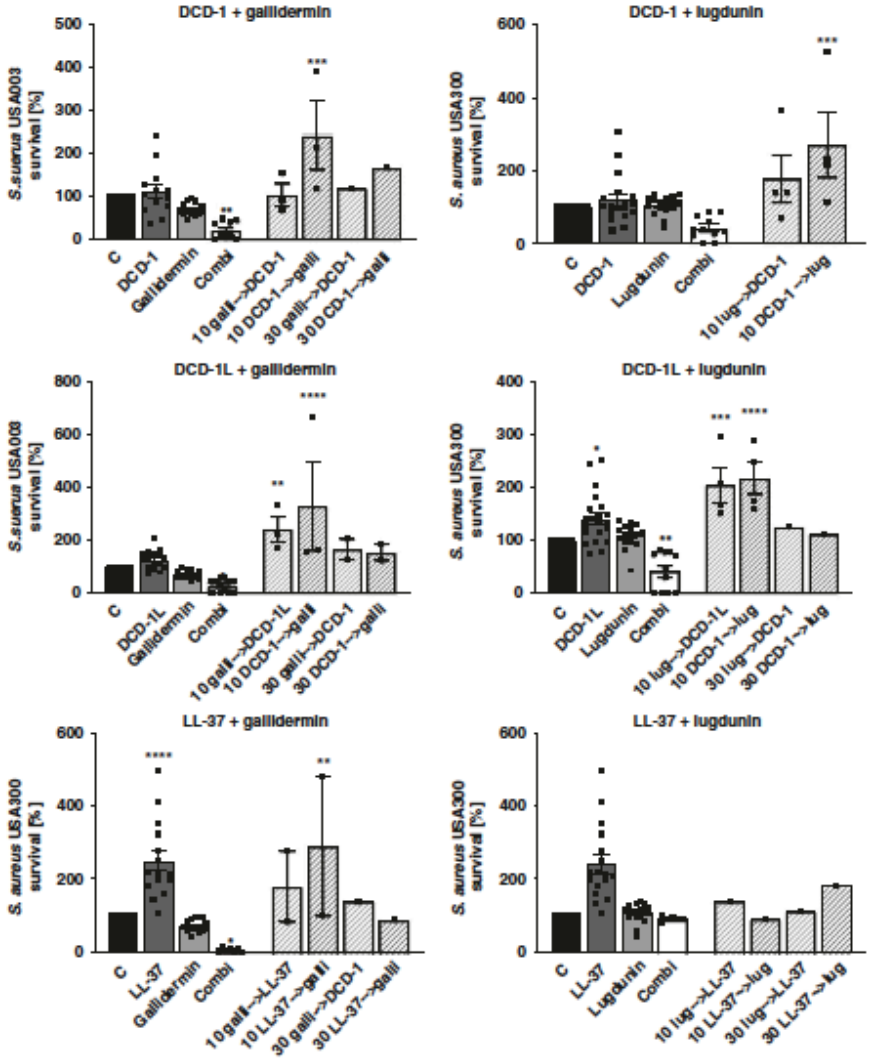


Fig. 6 Simultaneous treatment of peptides exhibits antimicrobial activity. Logarithmically grown (3×10^6) *S. aureus* were simultaneously (filled bars; combination treatment (Combi), white bars) or sequentially (striped bars) incubated with 2 µM of human antimicrobial peptides (AMPs) (dark gray bars), 2 µM lugdunin, and 0.8 µM gallidermin (both light gray bars) diluted in phosphate-buffered saline (PBS) containing 0.1% tryptic soy broth (TSB) in a 96-well V-plate. After 10 or 30 min incubation with the first single peptide at 37 °C and orbital shaking, bacteria were collected via centrifugation and were resuspended in a dilution containing the second peptide for 2 h and 50 min, or 2 h and 30 min, respectively. Several dilutions of the bacterial suspensions were plated onto TSB agar plates and incubated overnight at 37 °C. The next day *S. aureus* CFUs were counted. Each experiment was performed in triplicates. Data represent the percentage of CFU normalized to the untreated control ± s.e.m. Significant differences to control treatments were analyzed by ordinary one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$). Source data are provided as a Source Data file

TLR2-activating mechanism. The latter could resemble the mechanism of staphylococcal PSMs, which were shown to mobilize TLR2-activating lipopeptides[16]. Interestingly, however, antimicrobial activity of lugdunin with its original thiazolidine heterocycle building block seems to be crucial for innate immune activation in keratinocytes since the inactive *N*-acetyl lugdunin did not induce CXCL8 expression.

Binding of CXCL8/MIP-2 to CXCR1/2 on neutrophils results in the rapid recruitment of these effector cells to the site of infection[18–20]. In fact, we found that application of lugdunin onto mouse skin results in the recruitment of neutrophils and monocytes only 24 h after topical application. Therefore, clearance of pathogens by lugdunin-mediated recruitment of phagocytic cells complements its direct antimicrobial effects and thus provides an additional level of pathogen protection. The fact that a bacterial cyclic peptide can induce pro-inflammatory chemokine and AMP expression in epithelial cells as well as recruit immune cells to the skin is new and the detailed mechanisms still have to be elucidated in future experiments.

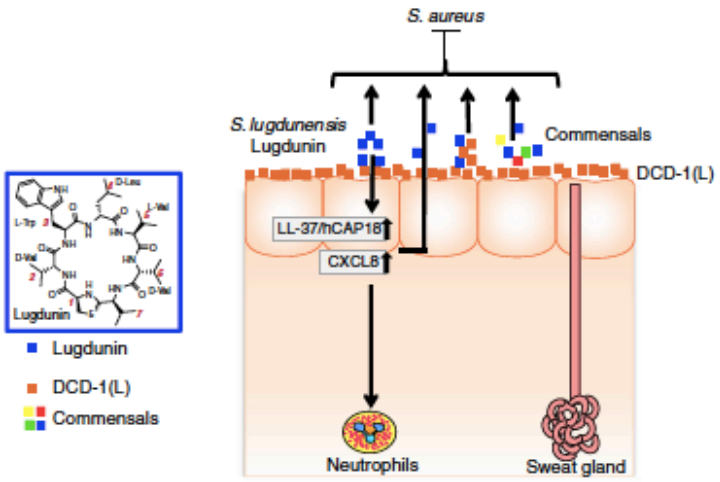


Fig. 7 Proposed model of lugdunin-mediated skin protection. Lugdunin acts on different levels to protect against *S. aureus* skin infection: First it can directly inhibit and kill *S. aureus*. Secondly, it can cooperate with host-derived antimicrobial peptides (AMPs) such as hCAP18/LL-37 and the dermcidin-derived peptides DCD-1(L) to kill *S. aureus*. Additionally, on a third level of protection, lugdunin induces an innate immune response of the skin, which leads to the recruitment of phagocytic immune cells, which will clear potentially invading pathogens. Finally, this innate immune response can be highly amplified by factors derived from the skin commensal *S. epidermidis*. Blue, lugdunin; orange, DCD-1(L); multicolored, commensals

In summary, the results of this study show that lugdunin provides multi-level protection of the host against *S. aureus* (Fig. 7). First, it can act synergistically with the human AMPs DCD-1(L) and LL-37 in killing MRSA. Secondly, lugdunin can amplify the commensal-induced innate immune response in PHKs. And last but not least, lugdunin-induced recruitment of phagocytic cells might additionally contribute to effective eradication of *S. aureus*. It has to be determined whether peptide antibiotics can be used to treat *S. aureus* skin infections in AD patients, but bacterial peptides and human AMP combination therapy may be a new option to combat MRSA skin infections through synergistic antimicrobial effects as well as enhancement of integral pathways of the cutaneous innate immune defense.

Methods

Bacterial strains, cells, and culture conditions. The Staphylococci used in this study were *S. aureus* USA300 LAC, *S. epidermidis* 1457, and *S. lugdunensis* IVK28 HR96. *Staphylococcus aureus* and *S. epidermidis* were aerobically grown in tryptic soy broth (TSB) and *S. lugdunensis* in basal medium (BM) at 37 °C and orbital shaking. The antimicrobial testing (AMT) assays were performed with logarithmically growing (optical density (OD) = 0.5) bacteria. Other bacterial strains used in this study were: *Pseudomonas aeruginosa* ATCC27853, *Proteus mirabilis* ATCC29906, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC19434, and *Bacillus subtilis* DB104. *Staphylococcus epidermidis* CM was generated by inoculating 25 mL keratinocyte CnT base medium (CELLnTEC) with 50 µL of an overnight *S. epidermidis* 1457 culture. After 18 h at 37 °C and orbital shaking (OD_{600} = 3), the culture was centrifuged and filter sterilized. Undiluted *S. epidermidis* CM was used in the following experiments.

Antimicrobial peptides. LL-37 and DCD-1/DCD-1L peptides were synthesized using Fmoc (9-fluorenylmethoxy carbonyl)/tBu chemistry with a multiple peptide synthesizer Syro II (MultiSynTech). After cleavage, peptides were purified by high-performance liquid chromatography (HPLC) on a reversed-phase C18 Nucleosil 100-5C column to a purity of >95% using a linear gradient of 5–80% acetonitrile in 0.05% trifluoroacetic acid for 45 min. Peptides were characterized by matrix-assisted laser desorption ionization-time of flight-mass spectroscopy and electrospray ionization and were in all cases in agreement with the calculated masses.[31,41] Nisin was purchased from Sigma (#N5764). Gallidermin and pro-gallidermin were isolated from a *Staphylococcus gallinarum* (F16/P57) Tü3928 culture by HCl extraction and reverse-phase HPLC purification[42]. Lugdunin was purified from a *S. lugdunensis* IVK28 culture by 1-butanol extraction, various washing steps, and preparative HPLC[10]. Additionally, lugdunin was synthesized by an Fmoc strategy-based manual solid-phase peptide synthesis[10]. *N*-acetylation of the thiazolidine heterocycle in lugdunin was achieved as follows: typically, 1 mg of lugdunin (1.3 µmol) was dissolved in 200 µL dimethyl sulfoxide. Approximately 100 equivalents of anhydrous sodium carbonate and 1.2 mL of acetic acid anhydride were added and the reaction mixture stirred at room temperature for 24 h. The reaction was quenched by the addition of excess H_2O. The crude reaction product was purified by standard preparative reversed-phase HPLC and afforded the product *N*-acetyl-lugdunin as a white solid in quantitative yields.

Antimicrobial testing. For bactericidal testing, logarithmically growing staphylococci were resuspended in PBS (Sigma) containing 0.1% TSB (Carl Roth) and colony-forming unit (CFU) was adjusted to 3×10^6 CFU/mL. Different concentrations of single peptides and their combinations were diluted in PBS containing 0.1% TSB and incubated with bacteria in triplicates for 3 h at 37 °C and 150 rpm orbital shaking. Subsequently, serial dilutions (10^{-1}–10^{-4}) of the bacterial suspensions were prepared in PBS and 20 µL of each dilution was spotted in duplicates onto TSB plates and incubated at 37 °C overnight. The next day, the number of CFU was analyzed and the percentage of viable bacteria was determined by normalizing to the untreated control (100%). Results are illustrated in a *S. aureus* killing curve. In each experiment, negative control replicates (PBS + 0.1% TSB) as well as sterility control replicates were included.

Sequential incubation of peptides. Logarithmically growing staphylococci were resuspended in PBS containing 0.1% TSB and CFU was adjusted to 3×10^6 CFU/mL. For sequential peptide incubation, the bacterial suspension was incubated with 2 µM of AMPs, 2 µM lugdunin, or 0.8 µM gallidermin diluted in PBS containing 0.1% TSB in a 96-well V-plate. After 10 or 30 min incubation with the first single peptide at 37 °C and orbital shaking, bacteria were collected via centrifugation for 5 min at 2000 rpm and bacteria were resuspended in a dilution containing the second peptide for 2 h and 50 min or 2 h and 30 min, respectively. The following steps were performed according to the AMT assay described above. In each experiment, combinations of the peptides and the single peptides in the respective concentrations were included as controls as well as sterility control replicates (PBS + 0.1% TSB).

Cell culture. PHKs and fibroblasts were isolated from human foreskin after routine circumcision from the Loretto Clinic in Tübingen upon informed consent of patients[6,43,44]. Keratinocyte and fibroblast isolation from human foreskin was approved by the ethics committee of the medical faculty of the University Tübingen (654/2014BO2) and performed according to the principles of the Declaration of Helsinki.

After removal of surplus fatty and vascular tissue, the foreskin was cut into small 1 cm^2 pieces and incubated overnight at 4 °C in epidermal keratinocyte medium with supplements (CELLnTEC) with 10 µg/mL gentamicin and 0.25 µg/mL amphotericin B (CELLnTEC) containing 10 mg/mL Dispase II (Roche) to digest the basal lamina. The next day, epidermis and dermis were carefully separated and small slices of the epidermis were incubated in 0.05% trypsin-EDTA (Merck Millipore) for 30 min, while small slices of the dermis were incubated in 1 mg/mL collagenase A (Roche) in fibroblast medium (CELLnTEC). Digestion was stopped using Roswell Park

Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS, Biochrom), and single cells were obtained using a 100-µm-pore-size cell strainer (Corning Incorporated). After centrifugation, cells were resuspended in epidermal keratinocyte medium with supplements (CELLnTEC) or fibroblast medium (CELLnTEC), respectively.

PHKs were cultured in collagen-coated tissue flasks (Corning, BioCoat™) in epidermal keratinocyte medium (CELLnTEC) at 37 °C, 5% CO_2[5,43,44]. Twenty-four hours prior to experiments, keratinocytes were differentiated with 1.7 mM $CaCl_2$ in epidermal keratinocyte base medium (CELLnTEC). Primary human fibroblasts were cultured in fibroblast medium (CELLnTEC). Primary human tracheal (HTEpCs) and nasal epithelial cells (HNEpCs) (PromoCell) were kindly provided by J. Schade (Interfaculty Institute of Microbiology and Infection Medicine Tübingen) and cultured in airway epithelial cell growth medium (PromoCell). HEK293 cells were cultured in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% FBS (Biochrom). For HEK293-TLR2 cells, 10 µg/mL normocin (InvivoGen) and 10 µg/mL blasticin (InvivoGen) were added to the culture medium. Before peptide stimulation fresh medium was added to cells. HEK293 and HEK293-TLR2 cells (InvivoGen) were kindly provided by D. Kretschmer (Interfaculty Institute of Microbiology and Infection Medicine Tübingen).

3D human skin equivalent. For 3D human skin equivalents, 1.35 mg/mL neutralized (pH 7.2–7.4) collagen I (Corning) was diluted in fibroblast medium (CELLnTEC) and 1 mL of collagen solution was added to 6-well inserts (0.4 µM, Merck). After 2 h of incubation at 37 °C, 8×10^5 fibroblasts diluted in 3 mL fibroblast medium were seeded on top of the collagen matrix. Subsequently, fibroblast medium was added to the bottom compartment of the insert. Dermal equivalents were incubated at 37 °C, and on days 2 and 4, fresh fibroblast medium was added. On day 5, 1×10^6 PHKs in 100 µL CnT (CELLnTEC) were seeded on top of the dermis. Concurrently, the medium in the bottom compartment was changed to CnT medium. From then on, the medium was changed every second day until day 12. From then on, skin equivalents were airlifted and the medium in the bottom compartment was changed to airlift medium (CELLnTEC). The medium was changed every second day until day 22.

On day 22, 1.5 µg lugdunin in 10 µL PBS were topically applied for 24 h onto the epidermis by using 8 mm filter paper discs (Smart Practice). The next day, the cell culture supernatant was used for ELISA (enzyme-linked immunosorbent assay)/Legendplex™.

Adhesion and invasion assay. Adhesion and invasion assays were performed by incubating differentiated PHKs with 2 µM lugdunin, *S. epidermidis*-CM or a combination of both or medium as a control for 18 h. The next day, keratinocyte supernatant was removed, keratinocytes were washed twice with Hank's balanced salt solution (HBSS) (Sigma), and fresh keratinocyte base medium containing 1.7 mM $CaCl_2$ was added. Subsequently, keratinocytes were infected with *S. aureus* (multiplicity of infection = 30; OD = 0.5) for 1.5 h. After two washing steps with HBSS, keratinocytes were lysed and serial dilutions of the lysates were plated onto blood agar plates. After overnight incubation at 37 °C CFUs were counted.

LEGENDplex™ multiplex cytokine analysis. For cytokine analysis from cell cultures, 10 µL of supernatant was used for cytokine analysis via the LEGENDplex™ human inflammation panel (BioLegend). For cytokine analysis from mouse skin, 4 mm skin punches were cultured in an airlift system where only the dermis had access to medium for 10 h. Skin punches were cultured in RPMI containing 1% FCS (Biochrom/Merck Millipore), 1% penicillin and streptomycin (Gibco/Life Technologies) and 0.25 µg/mL amphotericin B (CELLnTEC). Ten microliters of a 3-fold dilution of culture supernatant was used for cytokine analysis via the LEGENDplex™ mouse inflammation panel (BioLegend). Samples were acquired in duplicates using a BD LSRII flow cytometer (BD Biosciences) and LEGENDplex™ Software (BioLegend).

Enzyme-linked immunosorbent assay. For IL-1α analysis, 100 µL of cell culture supernatant was analyzed via ELISA (R&D Systems) according to manufacturer's instruction. For MIP-2 analysis, 100 µL of a 5-fold dilution from skin cultures supernatant was used and analyzed via ELISA (R&D Systems) according to the manufacturer's instruction. For LL-37 ELISA, ELISA plates (Nunc) were coated overnight at 4 °C with 100 µL of cell culture supernatant or 2-fold dilutions of LL-37 starting from 8 µg/mL. The next day, the plate was washed three times using PBS + 0.5% BSA + 0.05% Triton X-100, followed by incubation with 100 µL primary antibody against hCAP18/LL-37 (HycultBiotech, Cat#HM2071, 1:120 in PBS + 0.5% BSA + 0.05% Triton X-100) at 37 °C for 1 h. After washing, incubation with 100 µL secondary antibody (Cell Signaling, Cat#7076S, 1:3000 in PBS + 0.5% BSA + 0.05% Triton X-100) followed at 37 °C for 1 h. Subsequently, plates were washed and 100 µL TMB substrate solution (Cell Signaling) was added. Reaction was stopped with 50 µL 2 N H_2SO_4 and absorbance at 450 nm was measured using a Fluoroskan II (Labsystems).

Immunohistochemistry. For immunohistochemical stainings of mouse skin, 3 µM tissue sections were de-paraffinized. For MPO staining, antigen retrieval was performed in EDTA buffer at pH 9 (Thermo Fisher Scientific) for 5 min, and for MIP-2 and LL-37, citrate buffer at pH 6 for 9 min was used. Antigen retrieval was performed in a pressure cooker before a slow cooling down of the samples in the buffer. Afterwards, tissue sections were washed in PBS and blocked in 5% donkey serum in PBS containing 0.05% Triton X-100 for 90 min. For MPO staining, tissue sections were subsequently incubated overnight at 4 °C in a humid chamber with a MPO-specific antibody (R&D Systems, Cat#AF3667) diluted 1:50 in a blocking buffer. For MIP-2 and LL-37 staining, tissue sections were first incubated with primary enhancer (Lab Vision™ UltraVision™ LP Detection System, Thermo Fisher Scientific) for 20 min at room temperature, washed, and then incubated with AP polymer (Lab Vision™ UltraVision™ LP Detection System, Thermo Fisher Scientific) for 30 min at room temperature in a humid chamber, followed by incubation with the primary antibodies MIP-2 (Thermo Scientific, Cat#701126, 1:10 in blocking buffer) and LL-37 (Novus Biologicals, Cat#NB100-98689, 1:200 in blocking buffer) overnight at 4 °C in a humid chamber. The next day, tissue sections were washed and incubated with a 1:250 dilution of alkaline phosphatase-coupled secondary antibody (Novus Biologicals) for 90 min in a humid chamber at room temperature. After washing in PBS, staining was performed by using the Lab Vision™ liquid fast red substrate system (Thermo Fisher Scientific) according to the manufacturer's instructions. After washing in water, hematoxylin–eosin staining (Agilent/Dako) was performed for 2 min. After another washing step in water, tissue sections were mounted with Kaiser's glycerol gelatine (Merck).

RNA isolation and cDNA synthesis. After 5 h or 20 h of peptide treatment, PHKs were washed once with PBS, followed by the addition of RNA lysis buffer directly into the well. Total RNA was extracted using the Nucleospin RNA Kit (Macherey-Nagel) according to the manufacturer's protocol. Complementary DNA was synthesized using the Reverse-Transkriptase Kit (Thermo Scientific) with 2 µg of RNA, 4 µL of 5× RT buffer, 0.5 µL Maxima reverse transcriptase (200 U/mL), 1 µL of random hexamer primer (100 µM), dNTP (10 mM), and RNAse-free water to a total volume of 20 µL. After pre-incubation of RNA with water for 10 min at 70 °C, master mix was added and incubated for 10 min at 25 °C, followed by 45 min at 50 °C and a final heat inactivation step for 5 min at 85 °C.

Quantitative reverse transcription-polymerase chain reaction. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed in 10 µL reaction volume with SYBR™ Green PCR Master Mix (Thermo Fisher) according to the manufacturer's instructions using a LightCycler 96 (Roche Life Science). The initial denaturation step was at 95 °C for 5 min, followed by 40 cycles with 10 s each for the denaturation step at 95 °C, the annealing at individual temperature, and the elongation at 72 °C. Primer sequences and respective annealing temperatures are listed in Supplementary Table 1.

PBMC isolation. PBMC isolation from human blood was approved by the ethics committee of the medical faculty of the University of Tübingen (054/2017BO2).

Human PBMCs were isolated from the peripheral blood of healthy donors upon obtaining informed consent by Ficoll-Histopaque (Biochrom) gradient centrifugation. Cells were washed once in PBS and adjusted to a cell number of 1×10^6 mL^{-1} in RPMI-1640 medium (Gibco/Life Technologies) containing 10% PBS (Biochrom/Merck Millipore).

Viability assay. Effects of the used peptides on keratinocytes, PBMCs, and nasal and tracheal epithelial cell viability were tested using 4-methylumbelliferyl heptanoate (MUH). Briefly, cells were treated with peptides and respective peptide combinations for 24 h, followed by incubation with 100 µg/mL MUH (Sigma-Aldrich) in PBS for 1 h at 37 °C. The absolute fluorescence intensity at λ_{ex} of 355 nm and λ_{em} of 460 nm was measured using a Fluoroskan II (Labsystems).

Mouse model. All mouse experiments were conducted in accordance with the German regulations of the Gesellschaft für Versuchstierkunde/Society for Laboratory Animal Science (GV-SOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). All mouse experiments were approved (HT1/12; HT1/17) by the local authorities (Regierungspräsidium Tübingen). Animal studies were performed with 6–8-week-old female C57BL/6 WT/MyD88-ko/5xTLR-ko (TLR2$^{-/-}$; TLR3$^{-/-}$; TLR4$^{-/-}$; TLR7$^{-/-}$; TLR9$^{-/-}$) mice.

Mouse skin was shaved 3 days prior to experiments allowing potential micro wounds to heal and skin to recover from shaving. To analyze *S. aureus* skin colonization, 2 × 15 µL (right and left flank) containing 1.5 µg lugdunin, *S. epidermidis* CM, the combination of both, or PBS were epicutaneously applied for 24 h on the shaved back skin of C57BL/6 WT mice by using 8 mm filter paper discs and Finn Chambers (Smart Practice). The next day, Finn Chambers were removed and 2 × 15 µL of a bacterial suspension containing 1×10^8 *S. aureus* were epicutaneously applied using new filter paper discs and new Finn Chambers. After 24 h, mice were euthanized and 4 mm skin punches were used for *S. aureus* CFU analysis.

To analyze the immune cell composition and cytokines in the skin, 15 µL containing 1.5 µg lugdunin or PBS as a control were epicutaneously applied for 24 h on the shaved back skin of C57BL/6 WT/MYD88-ko/5xTLR-ko mice. After

24 h, mice were euthanized and relevant skin areas or 4 mm skin punches were removed for immune cell analysis and LEGENDplex™ (BioLegend) cytokine analysis.

Mouse immune cell isolation and staining procedure. To prepare single-cell suspensions, relevant dorsal skin area was transferred to PBS + 2% FBS (Biochrom/Merck Millipore). Subcutaneous fat was removed using a razor blade and skin tissue containing epidermal and dermal parts was transferred into a 2 mL reaction tube containing digestion solution. Digestion solution contained 0.05 mg/mL DNase I (Roche) and 0.25 mg/mL Liberase (Roche) in RPMI-1640 Medium (Gibco/Life technologies). After scissor-mediated tissue disintegration digestion was performed for 1 h at 37 °C and stopped by the addition of 100 µL of FCS (Biochrom/Merck Millipore). Single cells were separated by using an 80 µm cell strainer (Greiner Bio-One). After washing in PBS + 2% FBS, single-cell suspensions were treated with TruStain fcX™ anti-CD16/32 (1:50, BioLegend) and subsequently surface stained with the following monoclonal antibodies: CD45.2 (1:200, BioLegend, clone 104, Cat#109824), F4/80 (1:200, BioLegend, clone BM8, Cat#123110), CD11b (1:200, BioLegend, clone M1/70, Cat#101227), CD11c (1:200, BioLegend, clone N418, Cat#117337), Ly6G (1:200, BioLegend, clone 1A8, Cat#127614), Ly6C (1:200, BioLegend, clone HK1.4, Cat#128014), CD19 (1:200, BioLegend, clone 6D5, Cat#115508), CD3 (1:200, BioLegend, clone 17A2, Cat#100214), and NK1.1 (1:200, BioLegend, clone PK136, Cat#108714). Fixable viability dye eFluor520 (1:1000, eBioscience™) was used to exclude dead cells. All samples were acquired using a BD LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo (Treestar).

Quantification and statistical analysis. Significant differences between the means of the different treatments were evaluated using GraphPad Prism 7.0 (GraphPad Software, Inc.). Either unpaired, two-tailed Student's *t* test or one-way analysis of variance followed by Dunnett's multiple comparisons test was used for statistical analysis and indicated in the respective figure legends. Differences were considered statistically significant with a p value of <0.05. To evaluate potential synergistic effects of peptide combinations, the respective CIs were calculated using CompuSyn (ComboSyn Inc.) and indicated in median effect plots as a function of the bacteria fractions affected by the combinatorial peptide treatment. CI values of 1 indicate additive effects, whereas values <1 and >1 indicate synergistic and antagonistic effects, respectively. Data are visualized using GraphPad Prism 7.0 (GraphPad Software Inc.), MS Excel (Microsoft Corporation), or FlowJo (Treestar).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The authors declare that all the data that support the findings of this study are available from the corresponding author upon request. The source data underlying Figs. 1, 2a–e, 3b, d, 4, 5b, 5c, 6 and Supplementary Figures 1, 2a–c, 3, 4, 5 are provided as a Source Data file.

Received: 12 November 2018 Accepted: 21 May 2019
Published online: 21 June 2019

References

1. Nestle, F. O., Di Meglio, P., Qin, J. Z. & Nickoloff, B. J. Skin immune sentinels in health and disease. *Nat. Rev. Immunol.* **9**, 679–691 (2009).
2. Costello, E. K. et al. Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694–1697 (2009).
3. Oh, J. et al. Temporal stability of the human skin microbiome. *Cell* **165**, 854–866 (2016).
4. Kong, H. H. et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* **22**, 850–859 (2012).
5. Wertheim, H. F. et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* **5**, 751–762 (2005).
6. Burian, M., Bitschar, K., Dylus, B., Peschel, A. & Schittek, B. The protective effect of microbiota on *S. aureus* skin colonization depends on the integrity of the epithelial barrier. *J. Invest. Dermatol.* **137**, 976–979 (2017).
7. Iwase, T. et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature* **465**, 346–349 (2010).
8. Nakatsuji, T. et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci. Transl. Med.* **9**, eaah4680 (2017).
9. Bierbaum, G. et al. The biosynthesis of the lantibiotics epidermin, gallidermin, Pep5 and epilancin K7. *Antonie Van Leeuwenhoek* **69**, 119–127 (1996).
10. Zipperer, A. et al. Human commensals producing a novel antibiotic impair pathogen colonization. *Nature* **535**, 511–516 (2016).
11. Medzhitov, R. & Janeway, C. Jr. Innate immunity. *N. Engl. J. Med.* **343**, 338–344 (2000).
12. Schroder, J. M. & Harder, J. Antimicrobial skin peptides and proteins. *Cell Mol. Life Sci.* **63**, 469–486 (2006).
13. Wanke, I. et al. Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *J. Invest. Dermatol.* **131**, 382–390 (2011).
14. Fournier, B. The function of TLR2 during staphylococcal diseases. *Front. Cell Infect. Microbiol.* **2**, 167 (2012).
15. Bitschar, K., Wolz, C., Krismer, B., Peschel, A. & Schittek, B. Keratinocytes as sensors and central players in the immune defense against *Staphylococcus aureus* in the skin. *J. Dermatol. Sci.* **87**, 215–220 (2017).
16. Hanzelmann, D. et al. Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants. *Nat. Commun.* **7**, 12304 (2016).
17. Zlotnik, A. & Yoshie, O. Chemokines: a new classification system and their role in immunity. *Immunity* **12**, 121–127 (2000).
18. Krishna, S. & Miller, L. S. Host-pathogen interactions between the skin and Staphylococcus aureus. *Curr. Opin. Microbiol.* **15**, 28–35 (2012).
19. Miller, L. S. & Modlin, R. L. Toll-like receptors in the skin. *Semin. Immunopathol.* **29**, 15–26 (2007).
20. Rot, A. & von Andrian, U. H. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annu. Rev. Immunol.* **22**, 891–928 (2004).
21. Schittek, B. et al. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat. Immunol.* **2**, 1133–1137 (2001).
22. Rieg, S., Garbe, C., Sauer, B., Kalbacher, H. & Schittek, B. Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions. *Br. J. Dermatol.* **151**, 534–539 (2004).
23. Bieber, L. & Kahlmeter, G. Staphylococcus lugdunensis in several niches of the normal skin flora. *Clin. Microbiol. Infect.* **16**, 385–388 (2010).
24. Gong, J. Q. et al. Skin colonization by *Staphylococcus aureus* in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. *Br. J. Dermatol.* **155**, 680–687 (2006).
25. Dobson, A., Cotter, P. D., Ross, R. P. & Hill, C. Bacteriocin production: a probiotic trait? *Appl. Environ. Microbiol.* **78**, 1–6 (2012).
26. Kommineni, S. et al. Bacteriocin production augments niche competition by enterococci in the mammalian gastrointestinal tract. *Nature* **526**, 719–722 (2015).
27. Cogen, A. L. et al. 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).
28. Ebner, P. et al. Lantibiotic production is a burden for the producing staphylococci. *Sci. Rep.* **8**, 7471 (2018).
29. Janek, D., Zipperer, A., Kulik, A., Krismer, B. & Peschel, A. High frequency and diversity of antimicrobial activities produced by nasal staphylococcus strains against bacterial competitors. *PLoS Pathog.* **12**, e1005812 (2016).
30. Schittek, B., Paulmann, M., Senyurek, I. & Steffen, H. The role of antimicrobial peptides in human skin and in skin infectious diseases. *Infect. Disord. Drug Targets* **8**, 135–143 (2008).
31. Rieg, S. et al. Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J. Immunol.* **174**, 8003–8010 (2005).
32. Yamasaki, K. & Gallo, R. L. Antimicrobial peptides in human skin disease. *Eur. J. Dermatol.* **18**, 11–21 (2008).
33. Schittek, B. The antimicrobial skin barrier in patients with atopic dermatitis. *Curr. Probl. Dermatol.* **41**, 54–67 (2011).
34. Harder, J. et al. Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. *J. Invest. Dermatol.* **130**, 1355–1364 (2010).
35. Niyonsaba, F. et al. The human antimicrobial peptide dermcidin activates normal human keratinocytes. *Br. J. Dermatol.* **160**, 243–249 (2009).
36. Wang, E., Qiang, X., Li, J., Zhu, S. & Wang, P. The in Vitro immune-modulating properties of a sweat gland-derived antimicrobial peptide dermcidin. *Shock* **45**, 28–32 (2016).
37. Bengtsson, T., Lonn, J., Khalaf, H. & Palm, E. The lantibiotic gallidermin acts bactericidal against *Staphylococcus epidermidis* and Staphylococcus aureus and antagonizes the bacteria-induced proinflammatory responses in dermal fibroblasts. *Microbiologyopen* **7**, e00606 (2018).
38. Ryu, S., Song, P. I., Seo, C. H., Cheong, H. & Park, Y. Colonization and infection of the skin by *S. aureus*: immune system evasion and the response to cationic antimicrobial peptides. *Int. J. Mol. Sci.* **15**, 8753–8772 (2014).
39. Naik, S. et al. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. *Nature* **520**, 104–108 (2015).

40. Naik, S. et al. Compartmentalized control of skin immunity by resident commensals. *Science* 337, 1115–1119 (2012).
41. Steffen, H. et al. Naturally processed dermcidin-derived peptides do not permeabilize bacterial membranes and kill microorganisms irrespective of their charge. *Antimicrob. Agents Chemother.* 50, 2608–2620 (2006).
42. Kellner, R. et al. Gallidermin: a new lanthionine-containing polypeptide antibiotic. *Eur. J. Biochem.* 177, 53–59 (1988).
43. Nguyen, M. T. et al. Staphylococcal (phospho)lipases promote biofilm formation and host cell invasion. *Int. J. Med. Microbiol.* 308, 653–663 (2017).
44. Whiteley, L. et al. Entry, intracellular survival, and multinucleated-giant-cell-forming activity of *Burkholderia pseudomallei* in human primary phagocytic and nonphagocytic cells. *Infect. Immun.* 85, e00468–17 (2017).

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft with the funding grant numbers TR156/1 (B.Sch., A.P., F.C.K.), DFG510/8-1 and DFG510/8-2 (B.Sch., A.P.), RTG1708 (A.P., S.G., F.G.), by the German Center for Infection Research to A.P., the Institutional Strategy of the University of Tübingen (Deutsche Forschungsgemeinschaft, ZUK 63 to N.A.S.), and the Cluster of Excellence EXC2124.

Author contributions

K.B. and B.Sch. designed the experiments and wrote the manuscript, with critical input from A.P., B.K., F.G., F.C.K., H.K., S.G., M.K., N.A.S. and S.M. Animal experiments, immune cell isolation, multiplex cytokine analysis, viability assays, and ELISA were performed by K.B. Lugdunin was purified by M.K. and B.K. *N*-acetylation of lugdunin was done by M.K. and lugdunin was synthesized by N.A.S. Gallidermin and pro-gallidermin were purified by F.G. DCD and DCD-1L were synthesized by H.K. K.B., J.F. and H.D. performed the AMT assays. Adhesion and invasion assays and qRT-PCR were performed by K.B. and J.F. J.F. performed sequential peptide incubation assays. 3D skin equivalents and immunohistochemical stainings were exerted by B.Sa.

Additional information

Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-10646-7.

Competing interests: Eberhard Karls University Tübingen holds a patent for lugdunin (EP3072899B1).

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

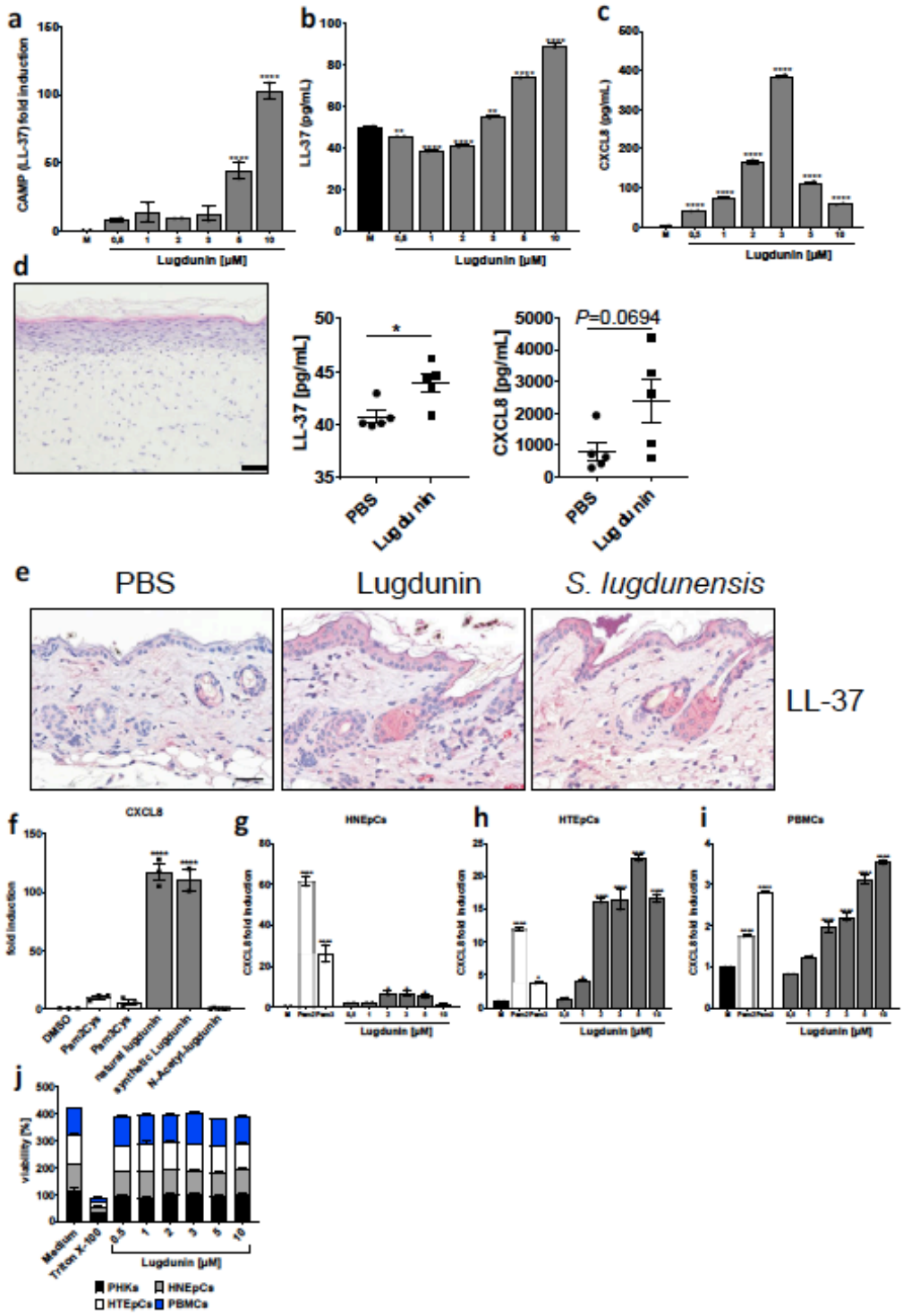
Peer review information: *Nature Communications* thanks Jens Schroeder and other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary Information

Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors

Bitschar et al.



Supplementary Figure 1: Lugdunin-induced LL-37 is concentration-dependent and CXCL8 is cell type-specific

a: PHKs were treated with increasing concentrations of lugdunin for 24 hours and subsequently expression of CAMP (LL-37) was analyzed and normalized to actin. Shown is one representative experiment of three independent experiments with two technical replicates each +/-s.e.m. Black bar: medium control.

b+c: PHKs were treated with increasing concentrations of lugdunin for 24 hours and subsequently the concentration of LL-37 and CXCL8 in the supernatant was analyzed. Shown is one representative experiment of three independent experiments, each with two technical replicates +/-s.e.m.

d: Representative hematoxylin-eosin-stained paraffin-embedded human 3D skin equivalent. Scale bar = 100 µM. 1.5 µg lugdunin or PBS was topically applied onto 3D skin equivalents. After 24 hours, the concentration of LL-37 and CXCL8 in the supernatant was analyzed. One dot represents one skin equivalent. Shown is the mean percentage +/- s.e.m. Significant differences to control treatments were analyzed by an unpaired two-tailed t-test (*P<0.05).

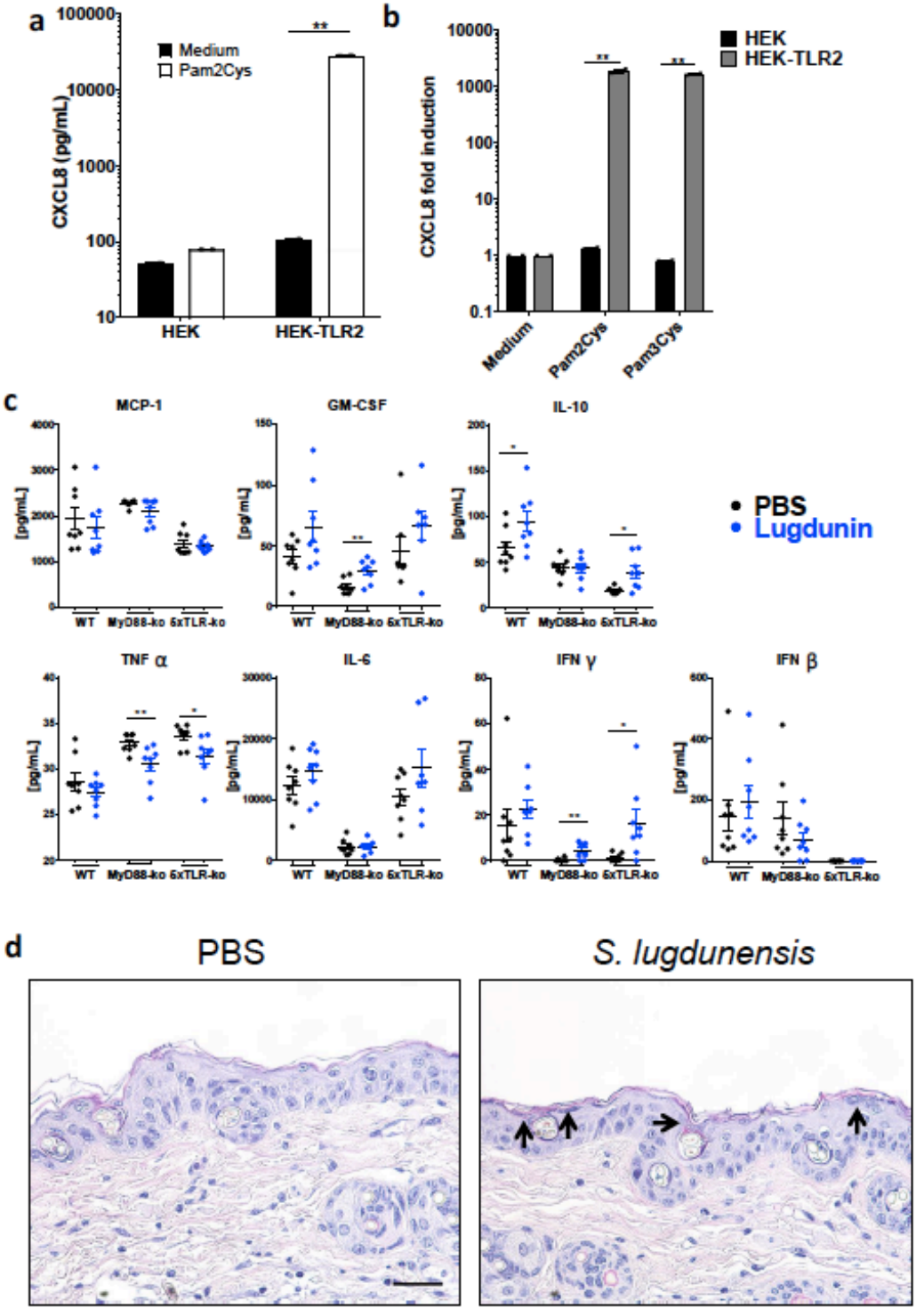
e: Representative LL-37-stained paraffin-embedded mouse skin sections. Scale bar = 100 µM.

f: PHKs were treated with 2 µM natural lugdunin, synthetic lugdunin, N-Acetyl lugdunin (grey bars) or 100 ng/mL Pam2Cys or Pam3Cys (white bars) as positive control for 5 hours and subsequently expression of CXCL8 was analyzed and normalized to actin. Black bar: DMSO control. Shown is one representative experiment of three independent experiments with at least two technical replicates +/-s.e.m.

g+h+i: HNEpCs (**b**), HTEpCs (**c**) or PBMCs (**d**) were treated with increasing concentrations of lugdunin (grey bars) or 100 ng/mL Pam2Cys or Pam3Cys (white bars) as positive controls for 5 hours and subsequently expression of CXCL8 was analyzed and normalized to actin. Black bar: medium control. Shown is one representative experiment of three independent experiments with two technical replicates +/-s.e.m. Significant differences to control treatments were analyzed by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

j: PHKs, HNEpCs, HTEpCs or PBMCs were treated with increasing concentrations of lugdunin or 0.1% Triton X-100 for 24 hours and subsequently incubated with 4-methylumbelliferyl heptanoate. Treatment with 0.1% Triton X-100 was used as negative control. Data were normalized to the untreated control. Shown is one

representative experiment of three independent experiments with two technical replicates +/-s.e.m. Source data are provided as a Source Data file.



Supplementary Figure 2: CXCL8 controls and other cytokines in mouse skin

a: HEK-control cells or HEK-TLR2 cells were treated with 100 ng/mL Pam2Cys for 5 hours and subsequently the CXCL8 concentration in the supernatant was analyzed.

Shown is one representative experiment of three independent experiments with two technical replicates +/-s.e.m.

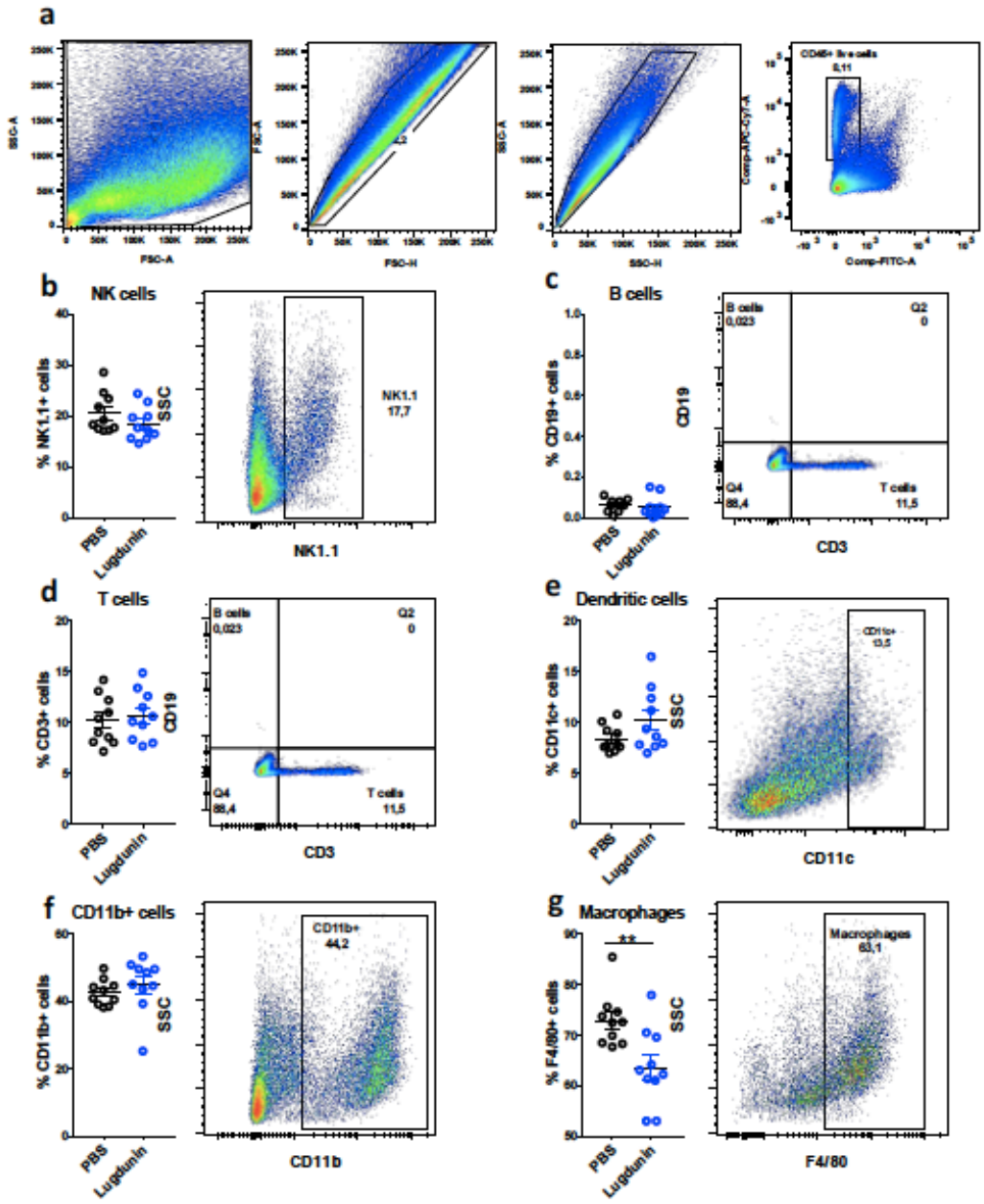
b: HEK-control cells or HEK-TLR2 cells were treated with 100 ng/mL Pam2Cys or Pam3Cys for 5 hours and subsequently expression of CXCL8 was analyzed. Shown is one representative experiment of three independent experiments with two technical replicates +/-s.e.m.

c: Shown are the mean concentrations of the indicated cytokines in the supernatant of the organ skin culture of two skin punches from four mice each +/- s.e.m.

Significant differences to control treatments were analyzed by an unpaired two-tailed t-test (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

d: Representative hematoxylin-eosin-stained paraffin-embedded mouse skin sections. Scale bar = 100 µM.

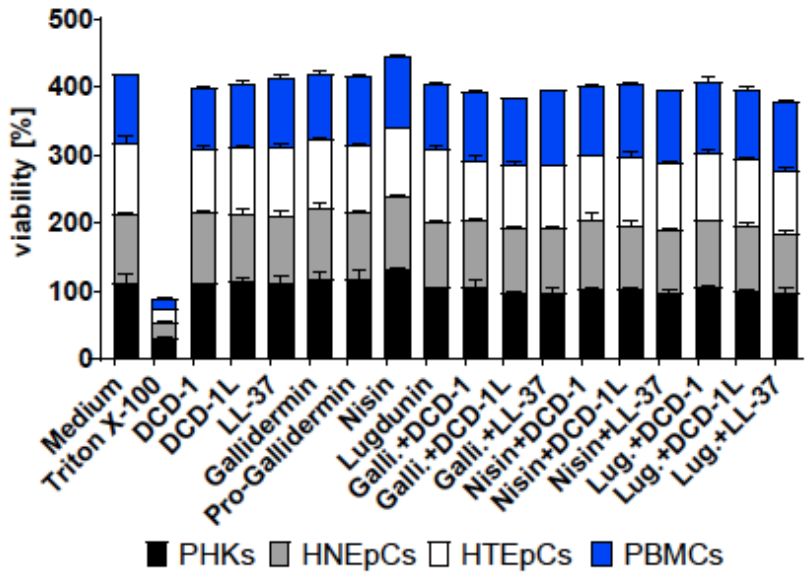
Source data are provided as a Source Data file.



Supplementary Figure 3: Gating strategy for immune cell analysis in mouse skin

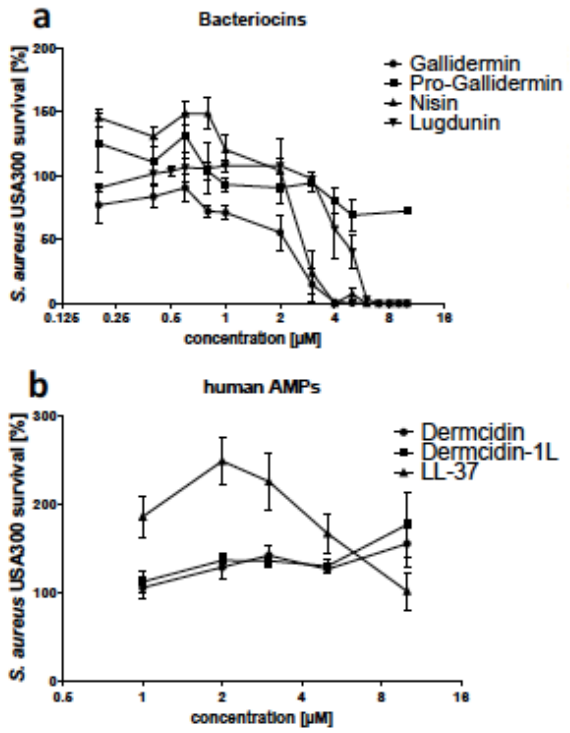
a: Gating strategy for live CD45+ cells. Gating for the different immune cell subsets pre-gated on live CD45+ cells: NK cells (**b**); B cells (**c**); T cells (**d**); Dendritic cells (**e**); CD11b+ cells (**f**); Pregated on CD11b+ CD45+ live cells presented on Figure 3d. Macrophages (**g**). Shown is the mean percentage of indicated immune cells in mouse skin of 10 C5BL/6 WT mice +/- s.e.m. One dot represents one mouse.

Source data are provided as a Source Data file.



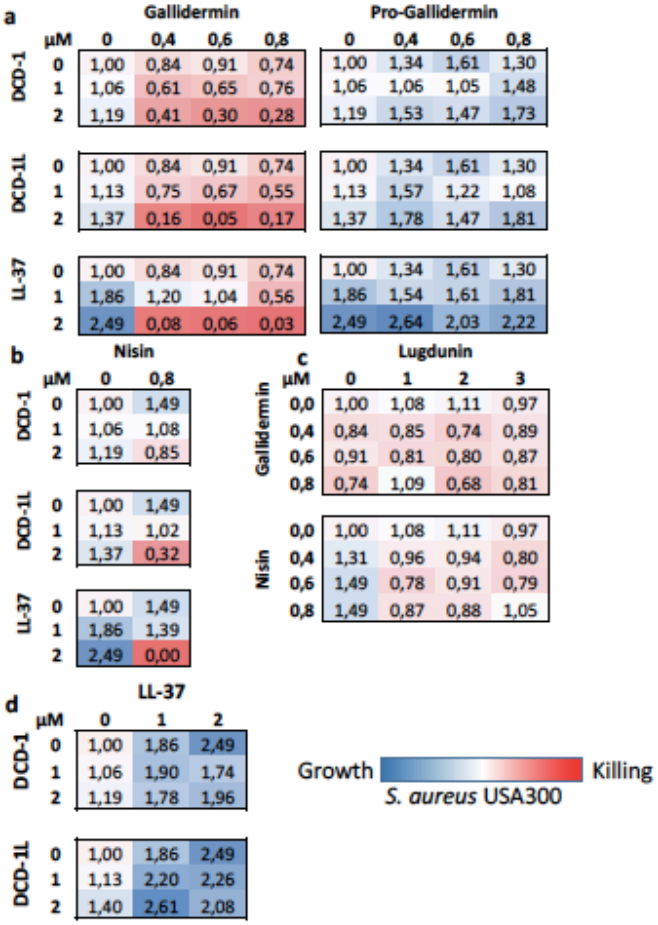
Supplementary Figure 4: Viability of primary cells upon AMP treatment

PHKS, HNEpCs, HTEpCs or PBMCs were treated with 2 μM human AMPs, 2 μM lugdunin, 0.8 μM of indicated bacteriocins or the correspondent peptide combinations for 24 hours. Subsequently, cells were incubated with 4-methylumbelliferyl heptanoate and viability was calculated. Treatment with 0.1 % Triton X-100 was used as negative control. Data were normalized to the untreated control. Shown is one representative experiment of three independent experiments with two technical replicates +/-s.e.m. Source data are provided as a Source Data file.



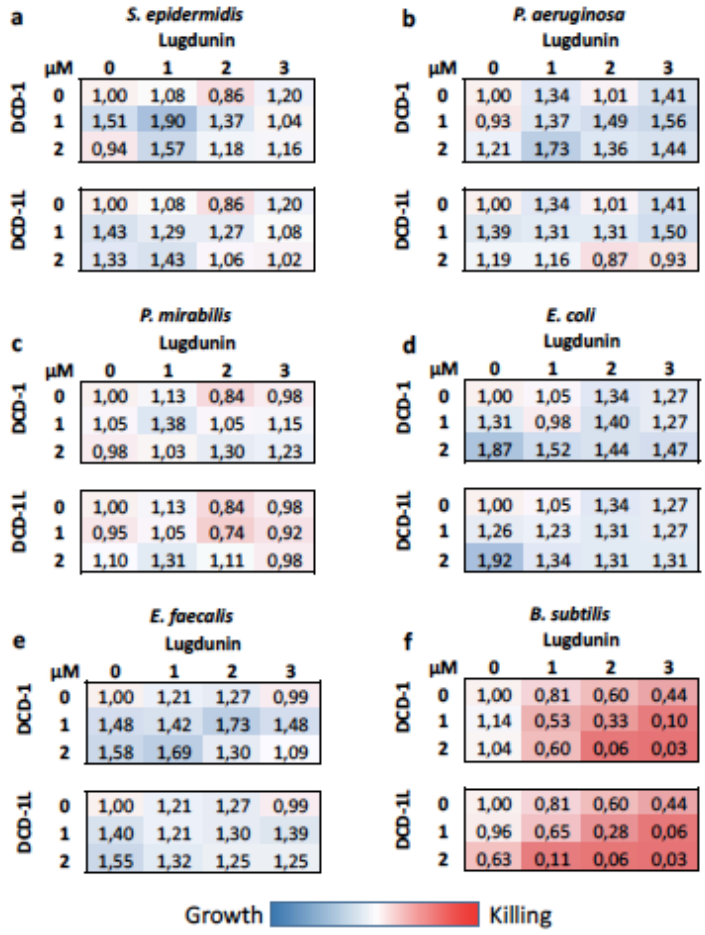
Supplementary Figure 5: Determination of minimal bactericidal concentrations of peptides used against *S. aureus* USA300

a+b: $3x10^6$ logarithmically grown *S. aureus* were incubated with indicated concentrations of the bacteriocins (pro)-gallidermin, nisin and lugdunin (**a**) or the human AMPs Dermcidin-1(L) and LL-37 (**b**) in PBS containing 0.1% TSB at 37 °C orbital shaking. After 3 hours of incubation several dilutions of the bacterial suspensions were plated onto TSB agar plates and incubated over night at 37 °C. The next day *S. aureus* CFU were counted. Each experiment was performed in triplicates. Data represent the mean percentage of *S. aureus* survival measured in CFU and normalized to the untreated control. Data represent the mean of at least three independent experiments +/- s.e.m. Source data are provided as a Source Data file.



Supplementary Figure 6: Combination treatments of *S. aureus* with different AMPs and bacteriocins

$3x10^6$ logarithmically grown *S. aureus* were incubated with indicated combinations of the bacteriocins (pro)-gallidermin (**a**) or nisin (**b**) with human AMPs or with combinations of lugdunin with gallidermin and nisin (**c**) or with combinations of DCD-1(L) and LL-37 (**d**) in PBS containing 0.1% TSB at 37 °C orbital shaking. After 3 hours of incubation several dilutions of the bacterial suspensions were plated onto TSB agar plates and incubated over night at 37 °C. The next day *S. aureus* CFU were counted. Each experiment was performed in triplicates. Data represent the mean percentage of CFU normalized to the untreated control. Data represent the mean of at least three independent experiments.



Supplementary Figure 7: Efficiency of Lugdunin and DCD-1(L) combinations in bacteria killing

$3x10^6$ logarithmically grown bacteria were incubated with indicated combinations of lugdunin and DCD-1(L) in PBS containing 0.1% TSB at 37 °C orbital shaking. After 3 hours of incubation several dilutions of the bacterial suspensions were plated onto TSB agar plates and incubated over night at 37 °C. The next day bacterial CFU were counted. Each experiment was performed in triplicates. Data represent the mean percentage of CFU normalized to the untreated control. Data represent the mean of at least three independent experiments.

116 **Supplementary Table 1: List of Primers used in this study**

Primer	Sequence	Annealing Temp
ACTB fw	TTGTTACAGGAAGTCCCTTGCC	60 °C
ACTB rv	ATGCTATCACCTCCCCTGTGTG	
CXCL8 fw	AGACAGCAGAGCACACAAGC	60 °C
CXCL8 rv	ATGGTTCCTTCCGGTGGT	
CAMP fw	TCGGATGCTAACCTCTACCG	58 °C
CAMP rv	GTCTGGGTCCCCATCCAT	
DEFB1 fw	TGTCTGAGATGGCCTCAGGT	60 °C
DEFB1 rv	GGGCAGGCAGAATAGAGACA	
DEFB4A fw	TCAGCCATGAGGGTCTTGTA	58 °C
DEFB4A rv	GGATCGCCTATACCACCAAA	
DEFB103A fw	TTCTGTTTGCTTTGCTCTTCC	62 °C
DEFB103 rv	CGCCTCTGACTCTGCAATAAT	
RNASE7 fw	GAAGACCAAGCGCAAAGC	58 °C
RNASE7 rv	CAGCAGAAGCAGCAGAAGG	

117

9.3 Accepted Publication III

Bitschar K., Wolz C., Krismer B., Peschel A. and Schittek B. Keratinocytes as sensors and central players in the immune defense against Staphylococcus aureus in the skin. J Dermatol Sci. 2017 Sep;87(3):215-220. doi: 10.1016/j.jdermsci.2017.06.003.

Journal of Dermatological Science 87 (2017) 215–220

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Review article

Keratinocytes as sensors and central players in the immune defense against *Staphylococcus aureus* in the skin

Katharina Bitschar[a], Christiane Wolz[b], Bernhard Krismer[c], Andreas Peschel[c], Birgit Schittek[a,*]

[a] Department of Dermatology, University of Tübingen, Tübingen, Germany
[b] Interfaculty Institute of Microbiology and Infection Medicine, Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany
[c] Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Tübingen, Germany

ARTICLE INFO

Article history:
Received 30 May 2017
Received in revised form 2 June 2017
Accepted 7 June 2017

Keywords:
Keratinocytes
Skin
Staphylococcus aureus
Atopic dermatitis
Immune response

ABSTRACT

Healthy human skin provides an effective mechanical as well as immunologic barrier against pathogenic microorganisms with keratinocytes as the main cell type in the epidermis actively participating and orchestrating the innate immune response of the skin. As constituent of the outermost layer encountering potential pathogens they have to sense signals from the environment and must be able to initiate a differential immune response to harmless commensals and harmful pathogens. Staphylococci are among the most abundant colonizers of the skin: Whereas *Staphylococcus epidermidis* is part of the skin microbiota and ubiquitously colonizes human skin, *Staphylococcus aureus* is only rarely found on healthy human skin, but frequently colonizes the skin of atopic dermatitis (AD) patients. This review highlights recent advances in understanding how keratinocytes as sessile innate immune cells orchestrate an effective defense against *S. aureus* in healthy skin and the mechanisms leading to an impaired keratinocyte function in AD patients.

Contents

Abbreviations: AMP, antimicrobial peptides; AD, atopic dermatitis; ECM, extracellular matrix; FPR2, formyl-peptide receptor 2; HMGB1, High-Mobility-Group-Box 1; LPP, lipopeptides; MAMP, microbe-associated molecular pattern; MyD88, myeloid differentiation factor 88; NRF2, nuclear factor erythroid 2–related factor 2; ROS, reactive oxygen species; RTK, receptor-tyrosine kinase; TLR, toll-like receptors; PSM, phenol-soluble modulins.

* Corresponding author at: Division of Dermatooncology, Department of Dermatology, University of Tübingen Liebermeister str. 25, Tübingen, D-72076, Germany.
E-mail address: birgit.schittek@uni-tuebingen.de (B. Schittek).

http://dx.doi.org/10.1016/j.jdermsci.2017.06.003
0923-1811/

1. Introduction

Skin is a unique ecologic niche that allows a diverse set of commensal microorganisms to colonize while at the same time it efficiently prevents pathogenic microorganisms from persisting. In this review we illustrate how keratinocytes, as the major cell type in the epidermis, initially sense invading pathogens as well as commensal microorganisms and how they orchestrate the subsequent innate immune response by producing antimicrobial peptides and cytokines. We further highlight the virulence factors of *S. aureus* that especially contribute to colonization of patients with epithelial barrier defects, whereas healthy skin does not seem to offer favorable conditions for *S. aureus* colonization.

2. Keratinocytes as initial sensors of infection

2.1. The sensing receptors

Keratinocytes are the main constituents in the epidermis – a continuously self-renewing epithelium, which consists of four distinct layers characterized by the differentiation status of the keratinocytes: the undifferentiated basal layer, the stratum spinosum, the further differentiated stratum granulosum and the stratum corneum with dead corneocytes. During their maturation process keratinocytes move from the basal to the uppermost layer and orchestrate immune responses if microbes and their molecules penetrate the stratum corneum upon mechanical or pathological barrier defects. Keratinocytes express several pattern recognition receptors (PRRs), which contribute to the initial sensing of microorganisms and intracellular signal transduction: Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I-like receptors (RLRs), and C-type lectin receptors (CLRs) [1,2]. TLRs are the best characterized human PRRs [3,4]. They recognize conserved microbial structures such as lipopolysaccaride (LPS), lipopeptides, peptidoglycan, flagellin or nucleic acids. Epidermal keratinocytes express the cell surface associated TLRs 1, 2, 4, 5 and 6 and the endosomal TLRs 3 and 9 [2,4]. In addition, TLR7 expression is induced through triggering of TLR3 by double-stranded RNA. The NOD receptors 1 and 2 are also expressed by human keratinocytes and are intracellular receptors that respond to bacterial peptidoglycan fragments. NOD2 responds mainly to peptidoglycan from Gram-positive bacteria such as *S. aureus*. NOD2 activation in the presence of TLR signals is especially effective in inducing an inflammatory response [5–7].

TLR2 is the predominant receptor recognizing staphylococcal ligands such as lipoproteins [7,8]. Mice deficient in TLR2 were shown to be highly susceptible to *S. aureus* systemic infections leading to sepsis [9]. Bacterial lipoproteins have a co-stimulating and synergistic effect with peptidoglycan, which is sensed by the intracellular PRRs NOD1 and NOD2 [8,10]. Interestingly, we could recently show that unsaturated fatty acids from skin are incorporated into bacterial lipoproteins and can increase TLR2 dependent immune stimulation [11]. In addition, it has recently been shown that phenol-soluble modulins (PSMs) produced by staphylococci can release lipoproteins from the bacterial cell envelope leading to increased immune stimulation of cells [12]. Additionally, the structure and degree of acylation of bacterial lipoproteins influences the consecutive immune response and the efficiency of *S. aureus* skin colonization and persistence [7].

TLRs and NOD receptors play a critical role in AD pathology: Responsiveness of TLR2 to *S. aureus* products seems to be lower in AD skin, which might be due to a TLR2 polymorphism (Arg753GLN) in some AD patients located within the intracellular part of the receptor diminishing intracellular signaling. This mutation correlated to increased skin infections with *S. aureus* and was associated with a more severe phenotype of AD [13,14]. Many AD patients express lower amounts of TLR2 in skin cells and produce less proinflammatory cytokines after TLR2 stimulation compared to healthy humans [7]. In addition to TLR2, also TLR9, NOD1 and NOD2 polymorphisms were described to correlate with an increased risk for AD [13,14]. This indicates that most likely innate immune sensing of microbial products, which are potentially involved in AD pathology, involves not just one TLR ligand but a whole array of potential ligands that signal via different PRRs transmitting different innate immune signals.

2.2. The intracellular signaling machinery

Binding of *S. aureus* lipoproteins to TLR2 homo- or heterodimers results in a whole cascade of signaling events translating via the intracellular adapter myeloid differentiation factor 88 (MyD88) in activation of NF-κB signaling and induction of proinflammatory cytokine and antimicrobial peptide (AMP) expression [3]. Activation of TLR receptors on human keratinocytes leads to a predominant TH1-type immune response and to the production of type-I interferons. The intracellular adaptor MyD88 is able to recruit several IL-1 receptor-associated kinases and activation of the MyD88-dependent pathway results in the induction of many genes that modulate NF-κB-dependent transcription and induction of proinflammatory cytokines [15]. These include ATF3, which restricts NF-κB activity by recruiting histone deacetylase [15] or the IκB protein NF-κBIζ (also called MAIL or IκBζ), which either enhances or suppresses transcription of NF-κB target genes depending on the cell type [16]. NF-κBIζ is constitutively expressed in keratinocytes, but expression can be enhanced by TLR ligands or IL-1. Interestingly, knockout of NF-κBIζ in mouse skin led to elevated levels of proinflammatory cytokines and chemokines and severe dermatitis resembling AD pathology [17]. These data suggest that NF-κBIζ is essential for homeostatic regulation of skin immunity.

The response of ligands binding to distinct TLRs must be stringently regulated to ensure appropriate immune and inflammatory responses. Interestingly, host and bacteria have evolved mechanisms that negatively regulate TLR and NF-κB signaling [18,19]. Expression levels of TLR2 and co-receptors regulate sensitivity to several TLR2 ligands. Soluble decoy TLRs, splice variants for adaptors or related proteins, ubiquitin ligases, transcriptional regulators such as ATF-3 and Stat1, and miRNAs can inhibit TLR and NF-κB signaling [15]. Ubiquitinylation represents an important regulatory mechanism of NF-κB signaling and pathogens evolved multiple mechanisms to exploit this posttranslational modification [20]. *S. aureus* infection of the skin imposes metabolic stress on keratinocytes that promotes HIF1α activation, glycolysis and a proinflammatory response and drives keratinocyte defense against *S. aureus* infection [21]. Epithelial barrier defects or dying cells could lead to the cleavage of extracellular matrix (ECM) components by cellular proteases or release of the DNA-binding protein High-Mobility-Group-Box 1 (HMGB1) and heat shock proteins, which are recognized by TLRs and could block activation of the respective TLR by exogenous ligands [15].

Furthermore, binding of ligands to TLRs can activate the transcription factor nuclear factor erythroid 2–related factor 2 (NRF2) in innate immune cells and epithelial cells such as keratinocytes. NRF2 plays a key role in skin homeostasis and repair and is involved in the stress response and defense against reactive oxygen species (ROS). It confers tissue damage control and protection against severe sepsis and *S. aureus* infections. It is suggested that keratinocytes depend on functional NRF2 to prevent excessive skin inflammation by inhibition of NF-κB signaling [22].

3. Keratinocytes as a source of proinflammatory cytokines and chemokines

Keratinocytes are important producers of pro- and anti-inflammatory mediators such as IL-1 family cytokines, IL-6, TNF, and IL-10 [23–25]. IL-1 family cytokines play a major role in skin barrier function and expression, cleavage, and secretion of these cytokines by keratinocytes are induced upon stimulation of PRRs [3,7]. Upon *S. aureus* stimulation of keratinocytes the inflammasome, a large intracellular multiprotein complex, activates caspase 1, which then generates active, processed IL-1β and IL-18, thus initiating a cascade of events resulting in production of IL-1α, TNF and IL-6 [23]. IL-36 cytokines, which also belong to the IL-1 cytokine family, are released by keratinocytes upon tissue injury or microbial stimulation. Interestingly, IL-36 cytokines are processed by proteases derived from recruited neutrophils [26]. Moreover, keratinocytes are important cellular sources of the T-cell growth factor IL-15 in the skin. Through the expression of CC-chemokine ligand 20 (Ccl20) keratinocytes can recruit regulatory T cells and Langerhans cell precursors to the skin. By expressing CXCL9, CXCL10, and CXCL11 activated keratinocytes attract different subtypes of T cells into the epidermis and thereby orchestrate skin inflammation [24]. Several studies showed that skin colonization with *S. aureus* in AD patients correlates with increased levels of proinflammatory cytokines and AD severity [7]. However, as a regulatory feedback mechanism, systemic immunosuppression is also induced by recruitment of myeloid-derived suppressor cells [27].

4. Keratinocytes as a source of antimicrobial peptides

In response to infection and inflammatory stimuli keratinocytes are able to produce several antimicrobial peptides (AMPs), also called host defense peptides, which show a broad spectrum of antimicrobial activity against a wide range of pathogens including bacteria, fungi, and enveloped viruses. In human skin, keratinocytes are a major source of AMPs and larger proteins such as the β-defensins HBD-1, HBD-2, and HBD-3, psoriasin, and RNase7. In addition to host defense, AMPs can also mediate chemotaxis of innate immune cells and thus induce the adaptive immune response against infections [2]. AMP expression can be induced by proinflammatory cytokines such as IL-1β, TNF-α, IL-17 and IL-22, after bacterial contact and during epidermal differentiation or wound healing [2]. In particular, IL-17A and IL-22, which are produced by TH17 cells, increase AMP production by keratinocytes [28]. By contrast, TH2 cytokines, which are overrepresented in AD skin, were shown to suppress AMP production by keratinocytes [2]. However, the levels of HBD-2, HBD-3, and RNAse7 were higher compared to healthy controls indicating that there is no defect in AMP production but AMP function might be impaired in AD patients [29].

It was shown that HBD-3 is both necessary and sufficient to account for the constitutive ability of human keratinocytes to kill *S. aureus* within minutes of contact with the cell surface [30]. Furthermore, a higher inducibility of HBD-3 is associated with a more favorable clinical course and outcome of *S. aureus* skin infections [31]. Two studies showed that a high baseline expression level of RNase7 confers protection against *S. aureus* infection of the skin, whereas there was no association with expression levels of HBD-2 and HBD-3 with skin infection [32,33]. The data suggest that limiting the extent of *S. aureus* skin colonization involves HBD-3 induction, while susceptibility to infection is substantially influenced by expression levels of RNase7.

5. Keratinocytes as a niche for skin commensals

Skin is a very special habitat for bacteria with challenging conditions that include dryness, low nutrient availability, high salt concentration, low pH, exposure to UV light, and presence of host antimicrobial peptides and lipids [24]. Nevertheless, human skin is populated by a complex microbiota whose composition is mainly determined by the ecologic feature of the body site indicating a crosstalk of host and microbiota [34,35].

Apart from occupying space the microbiota shields our skin from pathogen colonization by the release of antimicrobial peptides called bacteriocins that can directly act on pathogens. Commensal-produced factors were shown to inhibit *S. aureus* epithelial colonization [36,37]. Intriguingly, recent evidence shows that antimicrobial peptide-producing coagulase-negative staphylococci can be missing in AD and recolonization of skin of AD patients with antimicrobial-producing *S. epidermidis* and *S. hominis* significantly reduced *S. aureus* levels already 24 h after application [38].

Commensal microbes can also directly act on keratinocytes by releasing a collection of immune-modulatory molecules with tolerogenic or proinflammatory properties [35]. We found that *S. epidermidis* and its secreted factors elicit a protective immune response in keratinocytes preventing *S. aureus* skin colonization, which is, however, dependent on the integrity of the epithelial barrier [39–41]. Lipopeptides isolated from *S. epidermidis* are able to induce TLR2-mediated HBD-2 and HBD-3 expression in primary human keratinocytes and inhibit the growth of *S. aureus* [42]. TLR2-dependent induction of the miRNA-143 by *S. epidermidis* was shown to result in TLR2 downregulation [43]. This negative feedback loop of TLR2 induced by *S. epidermidis* might display a general regulatory mechanism of skin commensals to dampen microbial-induced TLR2 activation and concomitant proinflammatory signaling in keratinocytes. On the other hand, long-term commensal skin colonization seems to involve a crosstalk with dendritic cells and T cell subsets in deeper skin tissue [44,45]. Indeed, skin commensals were shown to penetrate the skin and reside within the dermis and the dermal adipose tissue of healthy human skin where they can directly and physically interact with host immune cells and influence the innate and adaptive immune system [46]. Characteristic changes in the composition of microbial consortia were associated with chronic skin disorders [47]. Interestingly, overabundance of cutaneous *S. aureus* especially during AD flares is associated with loss of microbiome diversity indicating that the skin microbiome shapes *S. aureus* skin colonization [48].

6. *S. aureus* colonization and virulence factors

S. aureus colonizes the moist skin of the anterior nares but is regularly found on other parts of the skin only if the skin barrier function is disturbed for instance by micro-lesions or in AD. *S. aureus* has many surface proteins mediating adhesion to cytokeratin, loricrin, or involucrin of corneocytes in the strateum corneum [49], but healthy skin does not appear to offer favorable conditions for persistent skin colonization. The reasons have remained elusive but it is likely that alterations in available nutrients and in skin microbiome composition associated with barrier defects are necessary for *S. aureus* to thrive on human skin [48]. Notably, *S. aureus* has potent virulence factors leading to infection of deeper skin tissues (e.g. in scalded-skin syndrome, impetigo, or furunculosis) or to extensive skin inflammation (e.g. in AD). The corresponding disintegrating and immunomodulatory *S. aureus* molecules are described below.

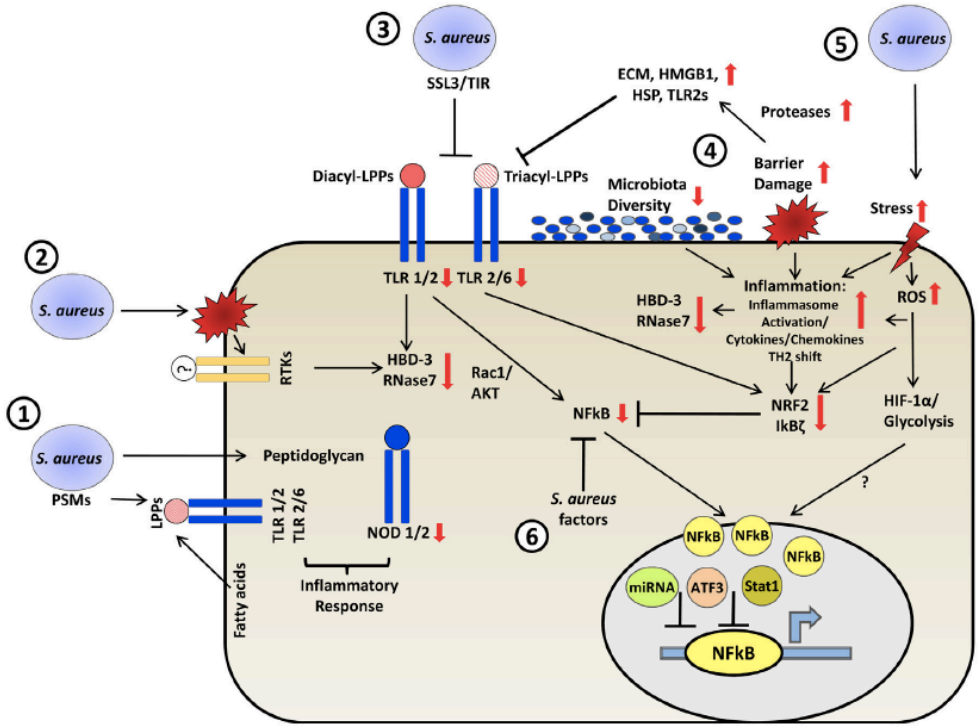


Fig. 1. Immune response of keratinocytes to *S. aureus*. Shown are the factors known to be involved in *S. aureus* skin colonization and infection. Red arrows indicate evidence for up- or down-regulation of the respective factor in *S. aureus* – infected skin of AD patients. Not shown is the influence of infiltrating immune cells on keratinocyte defense against *S. aureus*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
1: Staphylococcal peptidoglycan-induced NOD1/2 activation and PSM-mediated release of bacterial lipopeptides activating TLR2 are both able to induce a proinflammatory response. Unsaturated fatty acids from skin can be incorporated into bacterial lipoproteins and can increase TLR2 dependent immune stimulation.
2: Epithelial barrier disruption is able to stimulate HBD-3 and RNase7 expression by unknown ligands via transactivation of receptor tyrosine kinases (RTKs) as the EGFR. Skin inflammation and AMP induction is further amplified by *S. aureus* infection.
3: Diacylated and triacylated lipoproteins are recognized by TLR1/2 and TLR2/6 heterodimers, respectively. TLR2 heterodimers induce a proinflammatory host response via NF-κB activation and upregulation of AMPs. Staphylococcal virulence genes such as SSL3 or TIR counteract the lipopeptide-induced HBD-3 expression and NF-κB activation in order to escape from the host response.
4: Damage of the epithelial barrier for example during AD flares leads to overgrowth of *S. aureus* and subsequent loss of microbiome diversity, both leading to further enhancement of skin inflammation.
5: *S. aureus* imposes hypoxic stress on keratinocytes which induces HIF-1α in order to guarantee the metabolic demands for sufficient host defense.
6: *S. aureus* is able to circumvent host innate defense by various escape mechanisms, most of them targeting the NF-κB signaling axis in order to prevent host defense.

6.1. S. aureus *factors disintegrating skin tissues*

S. aureus produces a large array of proteins known to damage skin cells and skin integrity. The exfoliative toxin produced by certain lineages of *S. aureus* is a protease degrading the desmosomal protein desmoglein, thereby disrupting the cell-cell contacts between keratinocytes [50]. All *S. aureus* secrete up to ten other proteases some of which are known to affect the course of AD [51]. Staphylococcal phenol-soluble modulin (PSM) peptides have cytolytic activities at high concentrations [52]. One of the various *S. aureus* PSMs, the delta toxin, contributes to skin inflammation even at lower concentrations by activating mast cells [53]. Many furunculosis-inducing *S. aureus* produce the Panton-Valentine toxin, which damages leukocytes and keratinocytes [54]. The pore-forming α-toxin triggers inflammasome activation and cell death in human keratinocytes. The secreted *S. aureus* protein Eap alters the morphology, proliferation, and migration capacity of human keratinocytes in a currently unknown way [55]. Together, these aggressive virulence factors distinguish *S. aureus* from innocuous skin commensals and allow *S. aureus* to get access to deeper layers of the skin.

6.2. S. aureus *factors activating keratinocytes and skin immune cells*

S. aureus produces several microbe-associated molecular pattern (MAMP) molecules whose sensing by PRRs elicits inflammation contributing for instance to skin lesions in AD. Compared to skin commensals, *S. aureus* releases particularly high concentrations of bacterial lipoproteins, potent agonists of TLR2 on skin keratinocytes and leukocytes [12]. AD is associated with TLR2 polymorphisms in some patients, which supports the notion that *S. aureus* lipoproteins are major proinflammatory agonists in AD [7]. PSMs have proinflammatory activities at nanomolar concentrations [52,56] by stimulation of the formyl-peptide receptor 2 (FPR2), which is expressed by different types of immune cells and keratinocytes [52]. Moreover, PSMs mobilize the release of lipoproteins from the *S. aureus* cytoplasmic membrane thereby promoting TLR2 activation [12]. Several other *S. aureus* molecules

such as the T-cell activating superantigen toxins contribute to *S. aureus*-mediated skin inflammation [57].

6.3. S. aureus *immune evasion*

S. aureus is able to circumvent host innate defense by various escape mechanisms, PRR inhibitors and resistance to antimicrobial host peptides. Skin CAMPs such as defensins and RNase7 have only moderate activity against staphylococci which reduce their affinity for CAMPs by modification of cell wall molecules with positively-charged amino acids [58]. Moreover, several of the *S. aureus* exoproteases can cleave CAMPs. *S. aureus* secretes the potent TLR2 antagonist staphylococcal superantigen-like protein 3 (SSL3), which prevents binding of bacterial lipoproteins to TLR2 [59,60]. Moreover, a *S. aureus* TIR domain protein blocks TLR2-mediated NF-κB signaling by sequestering MyD88 [61]. Many *S. aureus* produce the CHIPS and FLIPr proteins, which block the formyl peptide receptors FPR1 and 2, respectively [62]. Blockage of these receptors prevents attraction and activation of innate immune cells. A potential contribution of these proteins to *S. aureus* skin colonization and infection remains to be explored in the future.

7. Conclusions

S. aureus colonization and infection of the skin leads to a whole cascade of events resulting in a rapid innate immune response with keratinocytes in the epidermis as the initial sensors and orchestrators (Fig. 1). Keratinocytes must allow harmless commensals to colonize the skin while at the same time preventing the induction of skin inflammation in order to maintain a peaceful relationship and skin homeostasis. Recent evidence indicates that commensals condition the immune response of keratinocytes as well as immune cells to induce a protective immune response that prevents *S. aureus* from colonizing the skin. Both the host and the bacterial factors involved in protection as well as the role of the integrity of the epidermal barrier still have to be elucidated. Our skin is very efficient in protecting us from *S. aureus* skin infection by diverse mechanisms such as rapid induction of antimicrobial peptides or recruitment of innate immune cells. The mechanisms involved in skin protection can be studied in patients where these mechanisms do not operate accurately and thus are not able to prevent *S. aureus* from colonizing the skin. There are still many unanswered questions remaining to be addressed: It is not yet understood how *S. aureus* manages to bypass the high levels of AMPs on the skin of AD patients. It has to be determined whether impaired AMP function or bacterial resistance mechanisms to circumvent killing by AMPs might be involved in persistent colonization of *S. aureus* on AD skin. Moreover, elucidation of the mechanisms as well as the bacterial factors involved in preferentially colonizing inflamed over healthy skin might contribute to further treatment options for AD patients.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the German Research Council to BS (TRR156, SCHI510/8-1) and by grants from the German Research Council to AP (TRR34, TRR156, SFB766, SFB685, GRK1708, PE805/5-1) and from the German Center for Infection Research to BK and AP (TTU HAARBI).

References

[1] M. Pasparakis, I. Haase, F.O. Nestle, Mechanisms regulating skin immunity and inflammation, Nat. Rev. Immunol. 14 (5) (2014) 289–301.
[2] B. Schittek, The antimicrobial skin barrier in patients with atopic dermatitis, Curr. Probl. Dermatol. 41 (2011) 54–67.
[3] T. Kawasaki, T. Kawai, Toll-like receptor signaling pathways, Front. Immunol. 5 (2014) 461.
[4] L.S. Miller, R.L. Modlin, Toll-like receptors in the skin, Semin. Immunopathol. 29 (1) (2007) 15–26.
[5] T. Volz, M. Nega, J. Buschmann, S. Kaesler, E. Guenova, A. Peschel, M. Rocken, F. Gotz, T. Biedermann, Natural Staphylococcus aureus-derived peptidoglycan fragments activate NOD2 and act as potent costimulators of the innate immune system exclusively in the presence of TLR signals, FASEB J. 24 (10) (2010) 4089–4102.
[6] S.A. Roth, M. Simanski, F. Rademacher, L. Schroder, J. Harder, The pattern recognition receptor NOD2 mediates Staphylococcus aureus-induced IL-17C expression in keratinocytes, J. Invest. Dermatol. 134 (2) (2014) 374–380.
[7] Y. Skabytska, S. Kaesler, T. Volz, T. Biedermann, The role of innate immune signaling in the pathogenesis of atopic dermatitis and consequences for treatments, Semin. Immunopathol. 38 (1) (2016) 29–43.
[8] M.T. Nguyen, F. Gotz, Lipoproteins of gram-Positive bacteria: key players in the immune response and virulence, Microbiol. Mol. Biol. Rev. 80 (3) (2016) 891–903 (MMBR).
[9] O. Takeuchi, K. Hoshino, S. Akira, Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to Staphylococcus aureus infection, J. Immunol. (Baltimore, Md.: 1950) 165 (10) (2000) 5392–5396.
[10] D.J. Philpott, M.T. Sorbara, S.J. Robertson, K. Croitoru, S.E. Girardin, NOD proteins: regulators of inflammation in health and disease, Nat. Rev. Immunol. 14 (1) (2014) 9–23.
[11] M.T. Nguyen, D. Hanzelmann, T. Hartner, A. Peschel, F. Gotz, Skin-Specific unsaturated fatty acids boost the staphylococcus aureus innate immune response, Infect. Immun. 84 (1) (2015) 205–215.
[12] D. Hanzelmann, H.S. Joo, M. Franz-Wachtel, T. Hertlein, S. Stevanovic, B. Macek, C. Wolz, F. Gotz, M. Otto, D. Kretschmer, A. Peschel, Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants, Nat. Commun. 7 (2016) 12304.
[13] L. Bin, D.Y. Leung, Genetic and epigenetic studies of atopic dermatitis, Allergy Asthma Clin. Immunol. 12 (2016) 52.
[14] S.J. Brown, Molecular mechanisms in atopic eczema: insights gained from genetic studies, J. Pathol. 241 (2) (2017) 140–145.
[15] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Nat. Immunol. 11 (5) (2010) 373–384.
[16] M. Yamamoto, S. Yamazaki, S. Uematsu, S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Kuwata, O. Takeuchi, K. Takeshige, T. Saitoh, S. Yamaoka, N. Yamamoto, S. Yamamoto, T. Muta, K. Takeda, S. Akira, Regulation of Toll/IL-1-receptor-mediated gene expression by the inducible nuclear protein IkappaBzeta, Nature 430 (6996) (2004) 218–222.
[17] T. Shiina, A. Konno, T. Oonuma, H. Kitamura, K. Imaoka, N. Takeda, K. Todokoro, M. Morimatsu, Targeted disruption of MAIL, a nuclear IkappaB protein, leads to severe atopic dermatitis-like disease, J. Biol. Chem. 279 (53) (2004) 55493–55498.
[18] M.M. Rahman, G. McFadden, Modulation of NF-kappaB signalling by microbial pathogens, Nat. Rev. Microbiol. 9 (4) (2011) 291–306.
[19] M. Johannessen, F. Askarian, M. Sangvik, J.E. Sollid, Bacterial interference with canonical NFkappaB signalling, Microbiology (Reading, England) 159 (Pt 10) (2013) 2001–2013.
[20] L.E. Reddick, N.M. Alto, Bacteria fighting back: how pathogens target and subvert the host innate immune system, Mol. Cell 54 (2) (2014) 321–328.
[21] M. Wickersham, S. Wachtel, T. Wong Fok Lung, G. Soong, R. Jacquet, A. Richardson, D. Parker, A. Prince, Metabolic stress drives keratinocyte defenses against staphylococcus aureus infection, Cell Rep. 18 (11) (2017) 2742–2751.
[22] R.K. Thimmulappa, H. Lee, T. Rangasamy, S.P. Reddy, M. Yamamoto, T.W. Kensler, S. Biswal, Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis, J. Clin. Invest. 116 (4) (2006) 984–995.
[23] P. Di Meglio, G.K. Perera, F.O. Nestle, The multitasking organ: recent insights into skin immune function, Immunity 35 (6) (2011) 857–869.
[24] F.O. Nestle, P. Di Meglio, J.Z. Qin, B.J. Nickoloff, Skin immune sentinels in health and disease, Nat. Rev. Immunol. 9 (10) (2009) 679–691.
[25] L.S. Miller, R.L. Modlin, Human keratinocyte Toll-like receptors promote distinct immune responses, J. Invest. Dermatol. 127 (2) (2007) 262–263.
[26] C.M. Henry, G.P. Sullivan, D.M. Clancy, I.S. Afonina, D. Kulms, S.J. Martin, Neutrophil-derived proteases escalate inflammation through activation of IL-36 family cytokines, Cell Rep. 14 (4) (2016) 708–722.
[27] Y. Skabytska, F. Wolbing, C. Gunther, M. Koberle, S. Kaesler, K.M. Chen, E. Guenova, D. Demircioglu, W.E. Kempf, T. Volz, H.G. Rammensee, M. Schaller, M. Rocken, F. Gotz, T. Biedermann, Cutaneous innate immune sensing of Toll-like receptor 2–6 ligands suppresses T cell immunity by inducing myeloid-derived suppressor cells, Immunity 41 (5) (2014) 762–775.
[28] M.L. Clausen, T. Agner, Antimicrobial peptides, infections and the skin barrier, Curr. Probl. Dermatol. 49 (2016) 38–46.
[29] J. Harder, S. Dressel, M. Wittersheim, J. Cordes, U. Meyer-Hoffert, U. Mrowietz, R. Folster-Holst, E. Proksch, J.M. Schroder, T. Schwarz, R. Glaser, Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury, J. Invest. Dermatol. 130 (5) (2010) 1355–1364.

[30] K.O. Kisich, C.W. Carspecken, S. Fieve, M. Boguniewicz, D.Y. Leung, Defective killing of Staphylococcus aureus in atopic dermatitis is associated with reduced mobilization of human beta-defensin-3, J. Allergy Clin. Immunol. 122 (1) (2008) 62–68.
[31] P. Zanger, J. Holzer, R. Schleucher, H. Scherbaum, B. Schittek, S. Gabrysch, Severity of Staphylococcus aureus infection of the skin is associated with inducibility of human beta-defensin 3 but not human beta-defensin 2, Infect. Immun. 78 (7) (2010) 3112–3117.
[32] P. Zanger, J. Holzer, R. Schleucher, H. Steffen, B. Schittek, S. Gabrysch, Constitutive expression of the antimicrobial peptide RNase 7 is associated with Staphylococcus aureus infection of the skin, J. Infect. Dis. 200 (12) (2009) 1907–1915.
[33] M. Simanski, S. Dressel, R. Glaser, J. Harder, RNase 7 protects healthy skin from Staphylococcus aureus colonization, J. Invest. Dermatol. 130 (12) (2010) 2836–2838.
[34] E.A. Grice, H.H. Kong, S. Conlan, C.B. Deming, J. Davis, A.C. Young, 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 (New York, N. Y.) 324 (5931) (2009) 1190–1192.
[35] A. SanMiguel, E.A. Grice, Interactions between host factors and the skin microbiome, Cell. Mol. Life Sci.: CMLS 72 (8) (2015) 1499–1515.
[36] 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 (7296) (2010) 346–349.
[37] A. Zipperer, M.C. Konnerth, C. Laux, A. Berscheid, D. Janek, C. Weidenmaier, M. Burian, N.A. Schilling, C. Slavetinsky, M. Marschal, M. Willmann, H. Kalbacher, B. Schittek, H. Brotz-Oesterhelt, S. Grond, A. Peschel, B. Krismer, Human commensals producing a novel antibiotic impair pathogen colonization, Nature 535 (7613) (2016) 511–516.
[38] 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. Leung, R.L. Gallo, Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis, Sci. Transl. Med. 9 (378) (2017).
[39] I. Wanke, H. Steffen, C. Christ, B. Krismer, F. Gotz, 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 (2) (2011) 382–390.
[40] I. Wanke, Y. Skabytska, B. Kraft, A. Peschel, T. Biedermann, B. Schittek, Staphylococcus aureus skin colonization is promoted by barrier disruption and leads to local inflammation, Exp. Dermatol. 22 (2) (2013) 153–155.
[41] M. Burian, K. Bitschar, B. Dylus, A. Peschel, B. Schittek, The protective effect of microbiota on S. aureus skin colonization depends on the integrity of the epithelial barrier, J. Invest. Dermatol. 137 (4) (2017) 976–979.
[42] 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 (3) (2013) e58288.
[43] X. Xia, Z. Li, K. Liu, Y. Wu, D. Jiang, Y. Lai, Staphylococcal LTA-induced miR-143 inhibits propionibacterium acnes-Mediated inflammatory response in skin, J. Invest. Dermatol. 136 (3) (2016) 621–630.
[44] 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 520 (7545) (2015) 104–108.
[45] 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 (New York, N.Y.) 337 (6098) (2012) 1115–1119.
[46] T. Nakatsuji, H.I. Chiang, S.B. Jiang, H. Nagarajan, K. Zengler, R.L. Gallo, The microbiome extends to subepidermal compartments of normal skin, Nat. Commun. 4 (2013) 1431.
[47] Y. Belkaid, J.A. Segre, Dialogue between skin microbiota and immunity, Science (New York, N.Y.) 346 (6212) (2014) 954–959.
[48] H.H. Kong, J. Oh, C. Deming, S. Conlan, E.A. Grice, M.A. Beatson, E. Nomicos, E.C. Polley, H.D. Komarow, 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 (5) (2012) 850–859.
[49] C. Weidenmaier, C. Goerke, C. Wolz, Staphylococcus aureus determinants for nasal colonization, Trends Microbiol. 20 (5) (2012) 243–250.
[50] M. Amagai, N. Matsuyoshi, Z.H. Wang, C. Andl, J.R. Stanley, Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1, Nat. Med. 6 (11) (2000) 1275–1277.
[51] Y. Hirasawa, T. Takai, T. Nakamura, K. Mitsuishi, H. Gunawan, H. Suto, T. Ogawa, X.L. Wang, S. Ikeda, K. Okumura, H. Ogawa, Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction, J. Invest. Dermatol. 130 (2) (2010) 614–617.
[52] A. Peschel, M. Otto, Phenol-soluble modulins and staphylococcal infection, Nature Rev. Microbiol. 11 (10) (2013) 667–673.
[53] Y. Nakamura, J. Oscherwitz, K.B. Cease, S.M. Chan, R. Munoz-Planillo, M. Hasegawa, A.E. Villaruz, G.Y. Cheung, M.J. McGavin, J.B. Travers, M. Otto, N. Inohara, G. Nunez, Staphylococcus delta-toxin induces allergic skin disease by activating mast cells, Nature 503 (7476) (2013) 397–401.
[54] C.Y. Chi, C.C. Lin, I.C. Liao, Y.C. Yao, F.C. Shen, C.C. Liu, C.F. Lin, Panton-Valentine leukocidin facilitates the escape of Staphylococcus aureus from human keratinocyte endosomes and induces apoptosis, J. Infect. Dis. 209 (2) (2014) 224–235.
[55] J. Eisenbeis, H. Peisker, C.S. Backes, S. Bur, S. Holters, N. Thewes, M. Greiner, C. Junker, E.C. Schwarz, M. Hoth, K. Junker, K.T. Preissner, K. Jacobs, M. Herrmann, M. Bischoff, The extracellular adherence protein (Eap) of Staphylococcus aureus acts as a proliferation and migration repressing factor that alters the cell morphology of keratinocytes, Int. J. Med. Microbiol.: IJMM 307 (2) (2017) 116–125.
[56] R. Wang, K.R. Braughton, D. Kretschmer, T.H. Bach, S.Y. Queck, M. Li, A.D. Kennedy, D.W. Dorward, S.J. Klebanoff, A. Peschel, F.R. DeLeo, M. Otto, Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA, Nat. Med. 13 (12) (2007) 1510–1514.
[57] A.R. Spaulding, W. Salgado-Pabon, P.L. Kohler, A.R. Horswill, D.Y. Leung, P.M. Schlievert, Staphylococcal and streptococcal superantigen exotoxins, Clin. Microbiol. Rev. 26 (3) (2013) 422–447.
[58] A. Peschel, H.G. Sahl, The co-evolution of host cationic antimicrobial peptides and microbial resistance, Nat. Rev. Microbiol. 4 (7) (2006) 529–536.
[59] K.J. Koymans, L.J. Feitsma, T.H. Brondijk, P.C. Aerts, E. Lukkien, P. Lossl, K.P. van Kessel, C.J. de Haas, J.A. van Strijp, E.G. Huizinga, Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3), Proc. Natl. Acad. Sci. U. S. A. 112 (35) (2015) 11018–11023.
[60] R. Yokoyama, S. Itoh, G. Kamoshida, T. Takii, S. Fujii, T. Tsuji, K. Onozaki, Staphylococcal superantigen-like protein 3 binds to the Toll-like receptor 2 extracellular domain and inhibits cytokine production induced by Staphylococcus aureus, cell wall component, or lipopeptides in murine macrophages, Infect. Immun. 80 (8) (2012) 2816–2825.
[61] F. Askarian, N.M. van Sorge, M. Sangvik, F.C. Beasley, J.R. Henriksen, J.U. Sollid, J. A. van Strijp, V. Nizet, M. Johannessen, A Staphylococcus aureus TIR domain protein virulence factor blocks TLR2-mediated NF-kappaB signaling, J. Innate Immun. 6 (4) (2014) 485–498.
[62] D.A. Bloes, D. Kretschmer, A. Peschel, Enemy attraction: bacterial agonists for leukocyte chemotaxis receptors, Nature reviews. Microbiology 13 (2) (2015) 95–104.

Katharina Bitschar is a PhD student at the University Hospital Tübingen in the Department of Dermatology supervised by Prof. Dr. Birgit Schittek. Her research focuses on the influence of commensal staphylococci on a Staphylococcus aureus skin infection. Thereby she is especially interested in how keratinocytes, as initial sensors of microbes, differentially trigger an adequate immune response that either leads to commensal tolerance or pathogen defense.

9.4 Accepted Manuscript I

24-Oct-2019

RE: MS# JID-2019-0543.R3

TITLE: Staphylococcus aureus skin colonization is enhanced by the interaction of neutrophil extracellular traps with keratinocytes

Dear Dr. Schittek:

Your manuscript has been favorably reviewed and is accepted for publication in The Journal of Investigative Dermatology. We look forward to future submissions from you and your colleagues.

YOU WILL RECEIVE AN ADDITIONAL MESSAGE FROM THE EDITORIAL OFFICE WITHIN 1 WEEK REGARDING ANY SPECIAL REQUIREMENTS WE MAY HAVE BEFORE PUBLICATION. This may include the possibility of publishing some materials online-only, due to limited space in the Journal. Please wait for this message before submitting publication materials.

We are accepting this manuscript for publication on the condition that neither the substance of the manuscript nor the text or data in this manuscript have been published, accepted for publication, or submitted for publication elsewhere. In signing the copyright agreement, the authors agree with this condition.

Publication of this article remains at the discretion of the editor; if substantial issues (e.g., authorship disputes, failure to fulfill ethics publication requirements, etc.) arise during the publication process, the editor may withdraw acceptance.

It is the policy of the Journal that unique non-proprietary reagents described (e.g., cells, DNA, antibodies) and instruments created for surveys must be made freely available to qualified scientists. Only under this condition is the information useful to the scientific community, and only in this way can results be reproduced by other scientists.

PUBLICATION MATERIALS
Publication materials should be received within 2 weeks; any delay will result in a delay in the publication of your article. See important information below regarding publication materials.

MAKING YOUR WORK CITABLE WITHIN DAYS
JID publishes all Original Articles and Letters to the Editor four business days following the transmission of final publication materials to the publisher. The publication is a .pdf of the accepted article's text and figures, and it includes a covering page with a disclaimer, along with a watermark and headers, that identifies the article as an unedited version of a peer-reviewed manuscript that has been accepted for publication. The covering page also includes instructions on how the article should be cited. Once the copyedited, typeset, and proofed version is published online, the manuscript version of the article will be removed. Both versions bear the same digital object identifier (DOI), allowing articles to be referenced as soon as they appear online and ensuring that all references to the article will be consistent.

USING THE DOI TO CITE ARTICLES PUBLISHED AHEAD OF PRINT:
[Author(s)]. [Title]. Journal of Investigative Dermatology accepted article preview, [publication date]; doi:[DOI]

OPEN ACCESS
Authors of research articles may choose to publish their accepted articles open access (i.e., free to all readers, regardless of whether they have a subscription) online immediately upon publication. After publication materials have been transmitted to the publisher, authors will be asked whether they wish to publish the article under JID's immediate open access options. The cost of immediate open access publication is $3200 ($2600 for ESDR and SID members).

MEET THE INVESTIGATOR

JID has initiated a new Facebook feature called "Meet the Investigator", in which we hope to introduce the Dermatology community to up-and-coming investigators. We invite you to recommend one of your junior co-authors for this feature; to do so, please send your nomination by return email; this can be brief, but a sentence or two describing how this individual will impact investigative dermatology in the future would be welcome. Please also include the nominee's email address and CV. Nominations will be reviewed by our "Meet the Investigator" Editor, and at least one profile will be selected each month.

We are delighted to publish your work in the JID.

Sincerely,

Mark C. Udey, MD, PhD
Editor
Journal of Investigative Dermatology

Staphylococcus aureus skin colonization is enhanced by the interaction of neutrophil extracellular traps with keratinocytes

Katharina Bitschar[1], Lena Staudenmaier[1], Laura Klink[1], Jule Focken[1], Birgit Sauer[1], Birgit Fehrenbacher[2], Franziska Herster[3], Zsofia Bittner[3], Lisa Bleul[4], Martin Schaller[2], Christiane Wolz[4], Alexander N. R. Weber[3], Andreas Peschel[4,5] and Birgit Schittek[1*]

[1]Department of Dermatology, Division of Dermatooncology, University of Tübingen, Tübingen, Germany

[2]Electron-Microscopy, Department of Dermatology, University Hospital Tübingen, Tübingen, Germany

[3]Department of Immunology, University of Tübingen, Tübingen, Germany

[4]Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Tübingen, Germany

[5]German Centre for Infection Research (DZIF), Partner Site Tübingen, Tübingen, Germany

*correspondence: birgit.schittek@med.uni-tuebingen.de

Abstract

Staphylococcus aureus is a facultative pathogen found on skin and nasal surfaces. It is usually absent from the skin of healthy humans but frequently colonizes the skin of atopic dermatitis patients.

Here we investigate the functional role of neutrophils in the initial steps of *S. aureus* skin colonization and how skin commensals modulate the *S. aureus*-induced recruitment of neutrophils to the skin. By using an epicutaneous mouse skin colonization model we show that skin inflammation induced by tape-stripping leads to a rapid recruitment of neutrophils which correlates with enhanced *S. aureus* skin colonization. Interestingly, depletion of neutrophils *in vivo* reduces *S. aureus* colonization and *in vitro* co-culture of primary human keratinocytes with neutrophils promotes *S. aureus* adherence. We demonstrate that the interaction of neutrophil extracellular traps (NETs) with keratinocytes are responsible for increased *S. aureus* skin colonization. Finally, we show that *S. epidermidis* as part of the skin microbiota can reduce neutrophil recruitment induced by *S. aureus* infection. These data suggest that microbiota-mediated skin protection against *S. aureus* is dampened in an inflammatory environment in which NETs released by infiltrating neutrophils unexpectedly contribute to enhanced *S. aureus* skin colonization.

Running title: NET release enhances *S. aureus* skin colonization

Key words: *Staphylococcus aureus*, skin colonization, atopic dermatitis, neutrophil extracellular traps, commensals

Abbreviations: atopic dermatitis (AD), conditioned medium (CM), methicillin-resistant *S. aureus* (MRSA), myeloperoxidase (MPO), neutrophil extracellular traps (NETs), peripheral blood mononuclear cells (PBMCs), phenol-soluble modulins (PSMs), polymorphonuclear cells (PMNs), primary human keratinocytes (PHKs)

Introduction

Human skin is populated by a complex microbiota which contributes to host protection against pathogens such as *S. aureus* in manifold ways (Grice et al., 2009, SanMiguel and Grice, 2015). While *S. aureus* is usually not present on healthy human skin it can be isolated from the anterior nares of 30% of the human population (Wertheim et al., 2005). Additionally, *S. aureus* colonization can become abundant on inflamed and non-inflamed skin of atopic dermatitis (AD) patients where it further contributes to skin inflammation (Eyerich et al., 2015, Geoghegan et al., 2018, Kong et al., 2012). Skin barrier defects in AD are multifactorial and it remains unclear which aspect primarily drives skin inflammation and *S. aureus* skin colonization (Eyerich et al., 2015, Schittek, 2011). Antimicrobial treatment interfering with *S. aureus* colonization can reduce the severity of AD indicating that *S. aureus* is actively contributing to skin inflammation (Eyerich et al., 2015, Paller et al., 2019). Interestingly, overabundance of cutaneous *S. aureus,* especially during AD flares, is associated with loss of microbiome diversity suggesting that the skin microbiome shapes *S. aureus* skin colonization (Kong et al., 2012, Oh et al., 2016). Several lines of evidence point to a major role of microbiome composition as a driving force of human predisposition or resistance to *S. aureus* colonization (Janek et al., 2016, Paller et al., 2019). These data indicate that the microbiome as well as an inflammatory environment influences *S. aureus* skin colonization. However, a mechanism that explains the ability of defined commensals as well as skin inflammation to drive *S. aureus* skin colonization before the onset of AD is not well understood

In our previous experiments we found that repeated tape-stripping of mouse skin induces pro-inflammatory cytokines and results in enhanced *S. aureus* skin colonization and persistence in an epicutaneous mouse skin colonization model

(Wanke et al., 2013). Furthermore, we found that *S. epidermidis* and its secreted factors elicit a protective immune response in keratinocytes in healthy mouse skin reducing *S. aureus* skin colonization, which is, however, reversed upon induction of skin inflammation by tape-stripping (Burian et al., 2017). These data indicate that skin inflammation modulates the microenvironment in a way that it can enhance pathogen colonization either by a crosstalk with skin-derived cells such as keratinocytes and/or by influencing the protective effect of the microbiome towards pathogen colonization.

In this work we investigate the mechanisms which contribute to initial *S. aureus* colonization during skin inflammation. Using a co-culture model of primary human keratinocytes and immune cells as well as an epicutaneous mouse skin colonization model we analyze the role of neutrophils during staphylococcal skin colonization.

Results

Skin inflammation induced by tape-stripping promotes *S. aureus* colonization *in vivo*

In our previous experiments we found that induction of skin inflammation by repeated tape-stripping of mouse skin results in enhanced *S. aureus* skin colonization and persistence in an epicutaneous mouse skin colonization model (Wanke et al., 2013). To identify the factors responsible for enhanced colonization of *S. aureus* on inflamed skin we confirmed the experiments using two different *S. aureus* strains - the virulent methicillin-resistant *S. aureus* (MRSA) strain USA300 and the non-virulent SA113 strain deficient in the *agr*-operon, a major virulence-controlling quorum-sensing system. Since both strains show increased colonization efficiency upon tape-stripping we conclude that this effect is independent of virulence factors (Figure 1a).

Next, we analyzed whether enhanced levels of pro-inflammatory cytokines induced by tape-stripping influence *S. aureus* skin colonization. Therefore, we analyzed the protein levels of several pro-inflammatory cytokines 24 hours after tape-stripping. We could show that tape-stripping leads to significantly changed levels of GM-CSF and IL-10 and clearly increased TNFα and IL-6 levels, which did not reach statistical significance (Figure 1b), whereas expression of IL-1α, MIP-2 (mouse homologue of CXCL8), MCP-1 and IFN-β was not significantly changed (Figure S1). To analyze whether this elevated pro-inflammatory environment contributes to enhanced *S. aureus* colonization, we treated primary human keratinocytes (PHKs) with a pro-inflammatory cytokine cocktail for 3 hours or overnight (data not shown), which highly induced gene expression of *IL1A*, *IL1B*, *TNFA*, *CXCL8* and *IL6* (Figure S1). Subsequently, we measured the number of *S. aureus* able to colonize PHKs using an established adhesion assay (Burian et al., 2017). However, pretreatment of PHKs with

this pro-inflammatory cocktail did not affect the number of PHK-colonizing *S. aureus* (Figure 1c). Therefore, we assumed that a pro-inflammatory cytokine environment alone did not directly affect *S. aureus* skin colonization and that other factors present *in vivo* influence the enhanced skin colonization after tape-stripping.

Tape-stripping induces enhanced neutrophil recruitment to the skin

The composition of different immune cell subsets recruited to the skin during the first hours after tape-stripping has not been characterized until now. Therefore, we analyzed the composition of different types of immune cells recruited to the skin 24 hours after tape-stripping (Figure 2a and Figure S2). We found that only percentages of neutrophils (live CD45+ CD11b+ Ly6G+ Ly6C+) and monocytes (live CD45+ CD11b+ Ly6C+) are significantly increased (Figure 2a and Figure S2) after tape-stripping, whereas percentages of overall $CD45^+$ and other immune cell subsets are only affected to a minor extent (Figure S2). The enhanced recruitment of neutrophils can also be seen in the immunohistochemistry staining for myeloperoxidase (MPO)-positive cells in mouse skin (Figure 2b).

Depletion of neutrophils *in vivo* reduces *S. aureus* skin colonization

The enhanced neutrophil recruitment in tape-stripped skin suggested that they might contribute to elevated *S. aureus* skin colonization. To test this idea, we depleted neutrophils in C57BL/6 wild-type mice by a single injection of an anti-Ly6G antibody 24 hours prior to skin colonization with *S. aureus* USA300 (Figure 3). Figure 3a shows that neutrophils were effectively depleted in all mice using the antibody treatment compared to the IgG control treatment. Impressively, neutrophil depletion resulted in a significant reduction of *S. aureus* skin colonization in most mice (Figure 3b). Even though a small subgroup of mice did not show reduced levels of *S. aureus*, these data clearly show that neutrophils contribute to enhanced *S. aureus* skin colonization.

Neutrophil recruitment in response to infected skin was shown to be dependent on IL-1β and the IL-1R1 (Miller and Modlin, 2007, Miller et al., 2006). Therefore, we used IL-1R1-ko mice as a model for deficient neutrophil recruitment. In fact, we could show that IL-1R1-deficient mice fail to recruit neutrophils in response to epicutaneous *S. aureus* colonization and interestingly, these mice already exhibit lower basal levels of neutrophils in healthy skin compared to WT mice (Figure 3c). Consistent with neutrophil depletion, we found that *S. aureus* colonization 24 hours after colonization of IL-1R1-deficient mice is significantly lower compared to WT mice (Figure 3d).

Neutrophils enhance *S. aureus* skin colonization independent of *S. aureus* virulence factors and lipopeptides

To further decipher the mechanism of the neutrophil-mediated elevation of *S. aureus* skin colonization we established an *in vitro* co-culture assay: PHKs were co-cultured with polymorphonuclear cells (PMNs) or peripheral blood mononuclear cells (PBMCs) for 18 hours using 0.4 µm cell culture inserts. Subsequently, PHKs were exposed to *S. aureus* and the number of viable colonizing bacteria 1.5 hours after infection was determined (Figure 4a).

Interestingly, we found that the presence of PMNs, but not PBMCs, significantly enhanced *S. aureus* adhesion to keratinocytes (Figure 4b). This effect is independent of direct PMN contact to the keratinocytes since they are separated by the insert (Figure 4b). Surprisingly, this effect could only be achieved when PMNs were pre-incubated with PHKs and were still present during the infection. Enhanced *S. aureus* colonization was not observed when pre-incubation was skipped or when PMNs were only present during the 1.5 hours of infection (Figure 4b and Figure S3). Additionally, the effect could neither be observed when PMN supernatant was used instead of living PMNs (Figure S3). These data suggest that soluble factors secreted by infected

keratinocytes induce PMNs to produce factors that create a staphylococcal-favoring environment. Interestingly, we could neither observe increased colonization with the non-virulent commensal strain *S. epidermidis* in the co-culture model nor in the *in vivo* model upon tape-stripping (Figure 4c, d). Therefore, we conclude that the neutrophil-mediated enhancement of skin colonization is specific for *S. aureus*.

Next, we wanted to identify the *S. aureus*-specific factors involved in neutrophil-mediated enhancement of staphylococcal skin colonization. We found similar levels of enhanced skin colonization *in vivo* of the virulent MRSA strain USA300 as well as with the non-virulent strain SA113 (see Figure 1a) suggesting that the effect is independent of *agr*-regulated virulence factors. However, to further analyze the influence of virulence factors and lipopeptides of *S. aureus* on the neutrophil-mediated enhancement of skin colonization, we used the USA300 Δ*agr*Δ*sae* double mutant (Munzenmayer et al., 2016), lacking virulence factors, and an USA300 Δ*lgt* mutant, lacking lipopeptides, in our co-culture model. However, we found that the neutrophil-mediated enhancement of skin colonization is completely independent of *S. aureus* virulence factors and lipopeptides (Figure 4e). This was confirmed *in vivo* using mice deficient in the pattern-recognition receptors (PRRs) MyD88, TLR2 and TLR9. Compared to WT control mice no enhanced skin colonization of *S. aureus* could be observed in these PRR-deficient mice upon tape-stripping indicating that these PRRs are not involved in enhanced *S. aureus* skin colonization in a neutrophil-rich environment (Figure 4f).

Released DNA is responsible for increased *S. aureus* skin colonization

Next, we wanted to identify the neutrophilic factors mediating this effect. To identify the substance class, we added DNAseI, protease inhibitors, phorbol-myristate-actetat (PMA), the calcium ionophore ionomycin or the formylated peptide N-formyl-met-leu-

189 phe (fMLF) to the PMN compartment (Figure S4). Interestingly, only DNAseI treatment
190 could reverse enhanced *S. aureus* colonization in the presence of PMNs (Figure 5a
191 and Figure S4). Protease inhibitors as well as the neutrophil-activating agents PMA,
192 ionomycin or fMLF did neither change nor further increase *S. aureus* skin colonization.
193 Treatment of PHKs only with the different substances did not show any effect on *S.*
194 *aureus* colonization in the absence of PMNs (Figure S4). These results suggested that
195 neutrophils release DNA which contributes to the enhancement of *S. aureus* skin
196 colonization.

197 **Neutrophil extracellular traps increase *S. aureus* skin colonization**

198 It is known that neutrophils can release neutrophil extracellular traps (NETs) in
199 response to a diverse set of stimuli including *S. aureus* infection. These NETs are
200 large, extracellular web-like structures which are released by neutrophils in order to
201 trap and kill microbes and consist of decondensed chromatin covered with
202 antimicrobial peptides (Branzk et al., 2014, Brinkmann et al., 2004, Papayannopoulos
203 and Zychlinsky, 2009).

204 We analyzed the presence of NETs in the co-culture system by SYTOX™ staining and
205 an anti-dsDNA ELISA. PMA stimulation of PMNs served as a positive control and
206 resulted in massive release of DNA in the SYTOX™ assay, which could be decreased
207 by DNaseI addition (Figure 5b). Interestingly, *S. aureus* infection of PHKs in our co-
208 culture setting also induced the release of DNA by PMNs although PMNs are not in
209 direct contact with the pathogen (Figure 5b). Furthermore, in line with our previous
210 results, extracellular DNA could not be observed when PHKs were infected with the
211 skin commensal *S. epidermidis* (Figure 5b). Using an anti-dsDNA ELISA we could
212 confirm that *S. aureus* infection of PHKs in the co-culture results in NET-formation by
213 neutrophils which could be reversed by DNAseI treatment (Figure 5c). To confirm

214 whether the extracellular DNA released by the neutrophils equals NETs, we performed
215 confocal laser scanning microscopy of neutrophils either directly infected with *S.*
216 *aureus* and *S. epidermidis* or in the co-culture system after infection of PHKs. PMA
217 treatment served as a positive control. Staining for citrullinated histone 3 (citH3) and
218 MPO, two characteristic NET components (Papayannopoulos, 2018), showed that
219 both direct *S. aureus* infection of neutrophils as well as *S. aureus* infection of PHKs in
220 the co-culture indeed result in NET formation whereas *S. epidermidis* infection did not
221 (Figure 5d). Finally, to ultimately prove that NETs increase *S. aureus* colonization of
222 PHKs we purified NETs from PMA-stimulated PMNs and co-cultured PHKs with NETs
223 only. In fact, we could observe a concentration-dependent increase of *S. aureus*
224 colonization which could be reversed by DNAseI addition (Figure 5e). Additionally, we
225 demonstrate that purified NETs did not exhibit cytotoxic effects on PHKs in the
226 concentrations tested (Figure 5f). Furthermore, in order to evaluate whether these
227 effects are exclusive for NETs we isolated genomic DNA (gDNA) from neutrophils and
228 added gDNA to the co-culture instead of PMNs. Indeed, *S. aureus* colonization of
229 PHKs could not be increased by gDNA indicating that the effect is specific for NETs
230 (Figure 5g). In addition to that, using the two compounds, GSK484 and Cl-Amidine,
231 that inhibit protein arginine deiminase 4 (PAD4), a critical player in NET formation
232 (Papayannopoulos, 2018), effectively prevented the release of extracellular DNA
233 (Supplementary Figure S4). These results confirm that NETs specifically lead to
234 enhanced *S. aureus* colonization of PHKs in the co-culture (Figure 5g). In summary,
235 our data show that soluble factors secreted by *S. aureus* infected keratinocytes induce
236 PMNs to release NETs, which influences PHKs in a yet unknown way that favors
237 colonization of *S. aureus*.

238

Skin commensals reduce *S. aureus*-induced neutrophil recruitment

Based on our previous results (Burian et al., 2017) we hypothesized that the microbiota might inhibit the recruitment of neutrophils induced by *S. aureus* and that under inflammatory conditions the *S. aureus* pro-colonizing effect of neutrophils dominates over the protective effect of the microbiome. Indeed, as shown in Figure 6a PHKs treated with *S. epidermidis* and its secreted factors (conditioned medium: CM) induce a lower and non-significant recruitment of PMNs in a Boyden Chamber migration assay compared to *S. aureus*-infected PHKs. Additionally, pretreatment of PHKs with *S. epidermidis* CM reduced the number of migrated PMNs in response to *S. aureus*-infected PHKs indicating that the microbiota modulates the release of neutrophil targeting chemokines and cytokines by PHKs (Figure 6a). Interestingly, we could also observe *in vivo* that *S. epidermidis* induces a lower recruitment of neutrophils and additionally reduces the *S. aureus*-induced neutrophil recruitment induced by *S. aureus* and might thus protect the skin from pathogen colonization (Figure 6b). This reduced neutrophil recruitment also correlated with a reduction in pro-inflammatory cytokines induced by *S. aureus* such as TNFα, IL-6, GM-CSF, IL-17A or IL-12p70 and others (Figure 6c). Other cytokines such as MCP-1, IL-10 and MIP-2 were not significantly changed by *S. epidermidis* pretreatment (Figure S6).

257 **Discussion**

258 Neutrophil migration to injured and infected tissues and concomitant clearance of
259 pathogens is a fundamental component of innate immunity (Kennedy and DeLeo,
260 2009, Verdrengh and Tarkowski, 1997). A multitude of signaling events including
261 secretion of neutrophil-recruiting chemokines and cytokines such as CXCL8 or IL-1 by
262 epithelial cells guide neutrophils from the bloodstream towards the infected or
263 damaged tissue.

264 It has previously been demonstrated by other groups that upon *S. aureus* infection of
265 the skin recruitment of neutrophils is critical in clearing the infection (Miller et al., 2006,
266 Molne et al., 2000). Yet, most existing data are based on subcutaneous or intradermal
267 *S. aureus* injection models. In a physiological setting, however, the first skin cells to
268 encounter invading pathogens are the keratinocytes (Bitschar et al., 2017). Thus,
269 keratinocytes need to orchestrate subsequent chemokine and cytokine production to
270 recruit neutrophils to the site of infection. Only recently, Liu et al. compared *S. aureus*
271 epicutaneous skin colonization to intradermal *S. aureus* injection and they found that
272 indeed critical players involved in *S. aureus* clearance upon bacterial injection have
273 adverse effects when *S. aureus* is epicutaneously applied (Liu et al., 2017). Here we
274 report the intriguing finding that enhanced presence of neutrophils in the skin of mice
275 or in a human *in vitro* co-culture system of keratinocytes and neutrophils enhances *S.*
276 *aureus* skin colonization.

277 We previously found that colonization as well as persistence of *S. aureus* on mouse
278 skin is significantly increased after induction of skin inflammation by tape-stripping
279 (Wanke et al., 2013). Here, we analyzed the mechanism of enhanced *S. aureus* skin
280 colonization and made the surprising observation that tape-stripping leads to the rapid

281 infiltration of neutrophils, which enhances *S. aureus* colonization by the release of
282 neutrophil extracellular traps (NETs).

283 We proved the involvement of neutrophils in *S. aureus* colonization by antibody-
284 mediated depletion of neutrophils *in vivo* as well as by supplementation of neutrophils
285 in a human *in vitro* co-culture assay with PHKs. Furthermore, addition of DNaseI in this
286 co-culture system reversed this enhancing effect and supplementation of isolated
287 NETs resulted in a similar *S. aureus* colonizing-enhancing effect, whereas inhibition of
288 NET formation by PAD4 inhibition prevented enhanced *S. aureus* colonization.
289 Interestingly, in the *in vitro* model neutrophils have to be present before *S. aureus*
290 infection, suggesting that there is already a crosstalk between neutrophils and
291 keratinocytes in inflamed skin before *S. aureus* infection which sensitizes to the NET-
292 induced effects. Future studies are necessary to resolve the mechanistic details in this
293 scenario.

294 Our observation is supported by the findings of Yipp et al. (2012): They used spinning-
295 disk confocal intravital microscopy to observe NET formation upon *S. aureus* skin
296 colonization. Disruption of these NETs by DNAseI treatment of mice resulted in
297 increased bacteremia and reduced *S. aureus* loads on the skin indicating that NETs
298 are important for containment and prevention of acute bloodstream infections but seem
299 counterproductive in skin infections (Yipp et al., 2012). These data suggest that NETs
300 are important to keep infections local in the initial stages of colonization. Future studies
301 should aim at analyzing how *S. aureus* uses NETs for increased colonization and
302 prevention of phagocytosis-mediated killing. Indeed, several mechanisms have been
303 described how *S. aureus* evades neutrophil-mediated killing (Bhattacharya et al., 2018,
304 Guerra et al., 2017).

Since it was recently shown that *S. aureus* Panton-Valentine leukocidine induces NETs (Pilsczek et al., 2010) we hypothesized that *S. aureus*-specific virulence factors are responsible for enhanced colonization. Additionally, bacterial-derived products such as N-formyl peptides or the phenol-soluble modulins (PSMs) produced by *S. aureus* have the demonstrated ability to directly recruit neutrophils, followed by activation and lysis (Wang et al., 2007). However, neither infection of PHKs with the Δ*agr*Δ*sae* double mutant, which lacks most virulence genes, nor infection with the lipoprotein-deficient mutant Δ*lgt*, reverted the increased *S. aureus* colonization in the presence of PMNs. In line with this we already observed that colonization with the *agr*-deficient SA113 strain is also increased upon tape-stripping. These results point to a *S. aureus*-specific mechanism which is independent of the investigated virulence factors.

The enhanced *S. aureus* skin colonization observed in this study resembles the elevated prevalence of *S. aureus* in patients suffering from atopic dermatitis (AD). AD is a multi-factorial disease with many factors such as genetic or environmental influences contributing to its pathogenesis (Eyerich et al., 2015). But until today the mechanisms that promote *S. aureus* colonization during the onset of disease and the role of the neutrophils during this process are unclear. Choy et al. (2012) compared the transcriptomic profile of AD skin to healthy skin and found that genes encoding for neutrophil chemoattractants such as CXCL8 or GM-CSF as well as the neutrophilic infiltrate in the dermis were significantly elevated in AD skin compared to healthy controls (Choy et al., 2012). In line with this, Dhingra et al. (2013) found that neutrophil-related genes such as DEFB4A or CXCL8 are significantly elevated in AD patients. Interestingly, they also associate higher neutrophil levels in AD with *S. aureus* infection (Dhingra et al., 2013). These data suggest that an enhanced presence of neutrophils

329 in the skin of AD patients contributes to the increased presence of *S. aureus*
330 colonization and persistence on AD skin.

331 We have recently demonstrated that *S. epidermidis* as part of the human skin
332 microbiome protects mouse skin from *S. aureus* colonization. However, induction of
333 skin inflammation by repeated tape-stripping inverted this protection and even
334 increased the colonization with *S. aureus* (Burian et al., 2017). Here, we demonstrate
335 that the commensal *S. epidermidis* is able to reduce PMN migration *in vitro* as well as
336 neutrophil recruitment *in vivo* which might partially explain why *S. epidermidis* is able
337 to protect the skin against *S. aureus* skin colonization (Burian et al., 2017). In line with
338 this, pretreatment with *S. epidermidis* also reduced the production of pro-inflammatory
339 cytokines in mouse skin.

340 In conclusion, this work shows that *S. aureus* benefits from the neutrophil-mediated
341 NET release which might contribute to the generation of microbial niches in inflamed
342 areas of the epithelial barrier. Even though NETs are considered important in the
343 antibacterial response, *S. aureus* seems to either misuse these structures for its own
344 advantage or it might be a safeguard mechanism which allows temporary *S. aureus*
345 colonization but by this might prevent the pathogen from entering deeper tissues.
346 Finally, we provide a model (graphical abstract) in which during skin homeostasis an
347 alliance of keratinocytes and the skin microbiota collectively prevents *S. aureus* from
348 colonizing the skin. However, upon colonization of skin with *S. aureus*, due to skin
349 inflammation or dysbiosis, neutrophils are recruited and undergo NET formation which
350 ultimately enhances *S. aureus* levels on the skin.

Materials and Methods

The detailed protocols, statistical analysis and ethics statements are described in Supplementary Materials and Methods online.

Bacterial strains and culture conditions

The Staphylococci used in this study were *S. aureus* USA300 LAC, *S. epidermidis* 1457 as well as the *S. aureus* USA300 mutants Δ*agr*Δ*sae* (Munzenmayer et al., 2016) and Δ*lgt*.

In vivo skin colonization model

Animal studies were performed with 6-8-week-old female C57BL/6 WT/IL-1R1/MyD88-ko/TLR2-ko/TLR9-ko mice. The in vivo skin colonization model was done as previously described (Bitschar et al., 2019, Burian et al., 2017, Zipperer et al., 2016).

Cell culture

Primary human keratinocytes (PHKs) were isolated from human foreskin after routine circumcision from the Loretto Clinic in Tübingen as previously described (Burian et al., 2017, Nguyen et al., 2017, Whiteley et al., 2017). 24 hours prior to experiments keratinocytes were differentiated with 1.7 mM $CaCl_2$ in epidermal keratinocyte base medium (CELLnTEC).

370 **Conflict of interest**

371 The authors declare no conflict of interest.

372 **Acknowledgements**

373 This work was supported by the Deutsche Forschungsgemeinschaft with the funding
374 grant numbers TR156/1 (B.S., A.P., A.W., C.W.), DFG510/8-2 (B.S., A.P.), RTG1708
375 (A.P.), the German Center for Infection Research to A.P and the Cluster of Excellence
376 EXC2124.

377

References

Bhattacharya M, Berends ETM, Chan R, Schwab E, Roy S, Sen CK, et al. Staphylococcus aureus biofilms release leukocidins to elicit extracellular trap formation and evade neutrophil-mediated killing. Proc Natl Acad Sci U S A 2018;115(28):7416-21.

Bitschar K, Sauer B, Focken J, Dehmer H, Moos S, Konnerth M, et al. Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. Nat Commun 2019;10(1):2730.

Bitschar K, Wolz C, Krismer B, Peschel A, Schittek B. Keratinocytes as sensors and central players in the immune defense against Staphylococcus aureus in the skin. J Dermatol Sci 2017;87(3):215-20.

Branzk N, Lubojemska A, Hardison SE, Wang Q, Gutierrez MG, Brown GD, et al. Neutrophils sense microbe size and selectively release neutrophil extracellular traps in response to large pathogens. Nat Immunol 2014;15(11):1017-25.

Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532-5.

Burian M, Bitschar K, Dylus B, Peschel A, Schittek B. The Protective Effect of Microbiota on S. aureus Skin Colonization Depends on the Integrity of the Epithelial Barrier. J Invest Dermatol 2017;137(4):976-9.

Choy DF, Hsu DK, Seshasayee D, Fung MA, Modrusan Z, Martin F, et al. Comparative transcriptomic analyses of atopic dermatitis and psoriasis reveal shared neutrophilic inflammation. J Allergy Clin Immunol 2012;130(6):1335-43 e5.

Dhingra N, Suarez-Farinas M, Fuentes-Duculan J, Gittler JK, Shemer A, Raz A, et al. Attenuated neutrophil axis in atopic dermatitis compared to psoriasis reflects TH17 pathway differences between these diseases. J Allergy Clin Immunol 2013;132(2):498-501 e3.

Eyerich K, Eyerich S, Biedermann T. The Multi-Modal Immune Pathogenesis of Atopic Eczema. Trends Immunol 2015;36(12):788-801.

Geoghegan JA, Irvine AD, Foster TJ. Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship. Trends Microbiol 2018;26(6):484-97.

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin microbiome. Science 2009;324(5931):1190-2.

Guerra FE, Borgogna TR, Patel DM, Sward EW, Voyich JM. Epic Immune Battles of History: Neutrophils vs. Staphylococcus aureus. Front Cell Infect Microbiol 2017;7:286.

Hashimoto M, Tawaratsumida K, Kariya H, Kiyohara A, Suda Y, Krikae F, et al. Not lipoteichoic acid but lipoproteins appear to be the dominant immunobiologically active compounds in Staphylococcus aureus. J Immunol 2006;177(5):3162-9.

Janek D, Zipperer A, Kulik A, Krismer B, Peschel A. High Frequency and Diversity of Antimicrobial Activities Produced by Nasal Staphylococcus Strains against Bacterial Competitors. PLoS Pathog 2016;12(8):e1005812.

Kennedy AD, DeLeo FR. Neutrophil apoptosis and the resolution of infection. Immunol Res 2009;43(1-3):25-61.

Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res 2012;22(5):850-9.

Liu H, Archer NK, Dillen CA, Wang Y, Ashbaugh AG, Ortines RV, et al. Staphylococcus aureus Epicutaneous Exposure Drives Skin Inflammation via IL-36-Mediated T Cell Responses. Cell Host Microbe 2017;22(5):653-66 e5.

Miller LS, Modlin RL. Toll-like receptors in the skin. Semin Immunopathol 2007;29(1):15-26.

Miller LS, O'Connell RM, Gutierrez MA, Pietras EM, Shahangian A, Gross CE, et al. MyD88 mediates neutrophil recruitment initiated by IL-1R but not TLR2 activation in immunity against Staphylococcus aureus. Immunity 2006;24(1):79-91.

Molne L, Verdrengh M, Tarkowski A. Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus. Infect Immun 2000;68(11):6162-7.

Munzenmayer L, Geiger T, Daiber E, Schulte B, Autenrieth SE, Fraunholz M, et al. Influence of Sae-regulated and Agr-regulated factors on the escape of Staphylococcus aureus from human macrophages. Cell Microbiol 2016;18(8):1172-83.

Nguyen MT, Luqman A, Bitschar K, Hertlein T, Dick J, Ohlsen K, et al. Staphylococcal (phospho)lipases promote biofilm formation and host cell invasion. Int J Med Microbiol 2017.

Oh J, Byrd AL, Park M, Program NCS, Kong HH, Segre JA. Temporal Stability of the Human Skin Microbiome. Cell 2016;165(4):854-66.

Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, et al. The microbiome in patients with atopic dermatitis. J Allergy Clin Immunol 2019;143(1):26-35.

Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 2018;18(2):134-47.

Papayannopoulos V, Zychlinsky A. NETs: a new strategy for using old weapons. Trends Immunol 2009;30(11):513-21.

Pilsczek FH, Salina D, Poon KK, Fahey C, Yipp BG, Sibley CD, et al. A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus. J Immunol 2010;185(12):7413-25.

SanMiguel A, Grice EA. Interactions between host factors and the skin microbiome. Cell Mol Life Sci 2015;72(8):1499-515.

Schittek B. The antimicrobial skin barrier in patients with atopic dermatitis. Curr Probl Dermatol 2011;41:54-67.

Verdrengh M, Tarkowski A. Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus. Infect Immun 1997;65(7):2517-21.

Wang R, Braughton KR, Kretschmer D, Bach TH, Queck SY, Li M, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 2007;13(12):1510-4.

Wanke I, Skabytska Y, Kraft B, Peschel A, Biedermann T, Schittek B. Staphylococcus aureus skin colonization is promoted by barrier disruption and leads to local inflammation. Exp Dermatol 2013;22(2):153-5.

Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis 2005;5(12):751-62.

Whiteley L, Meffert T, Haug M, Weidenmaier C, Hopf V, Bitschar K, et al. Entry, Intracellular Survival, and Multinucleated-Giant-Cell-Forming Activity of Burkholderia pseudomallei in Human Primary Phagocytic and Nonphagocytic Cells. Infect Immun 2017;85(10).

Yipp BG, Petri B, Salina D, Jenne CN, Scott BN, Zbytnuik LD, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. Nat Med 2012;18(9):1386-93.

Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, Weidenmaier C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. Nature 2016;535(7613):511-6.

Figure Legends

Figure 1: Skin inflammation increases *S. aureus* colonization *in vivo*

Dorsal skin of WT mice was either tape-stripped or left untreated. **a**: Subsequently, skin was epicutaneously infected with $1x10^8$ *S. aureus* SA113 or USA300. After 24 hours the number of *S. aureus* CFU colonizing the skin was determined. Horizontal lines represent the mean of each group +/- SEM. **b:** 4 mm skin punches were taken and further cultured *in vitro* for 10 hours followed by cytokine analysis of the culture supernatant by LEGENDplex™ (BioLegend). Shown are the mean concentrations of the indicated cytokines in the supernatant of the skin culture of one skin punch from six mice +/- SEM. **c**: Primary human keratinocytes (PHKs) were pretreated with a pro-inflammatory cytokine cocktail (10ng/mL IL-1α, TNFα, Oncostatin M, IL-22 and IL-17A) for 3 hours. Subsequently, cells were infected (MOI=30) with *S. aureus* USA300 for 1.5 hours followed by cell lysis. Serial dilutions of the lysate were spotted on blood agar plates and CFU were determined. Data represent the mean of four independent experiments normalized to their untreated control. Significant differences to control treatments were analyzed by an unpaired two-tailed t-test (**P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001). TS= Tape-stripping.

Figure 2: Tape-stripping induces neutrophil and monocyte recruitment to mouse skin

a+b: Dorsal skin of WT mice was either tape-stripped or left untreated. **a**: After 24 hours immune cells were isolated from treated skin areas and the immune cell composition was analyzed by flow cytometry. Shown is the mean percentage of neutrophils (Ly6C+ Ly6G+) and monocytes (Ly6C+) pre-gated on CD11b+ CD45+ live cells in mouse skin of 9 C5BL/6 WT mice +/- SEM. One dot represents one mouse. Significant differences to control treatments were analyzed by an unpaired two-tailed t-test (**P*<0.05). **b**:

498 Representative MPO-stained (myeloperoxidase) paraffin-embedded skin sections.
499 Scale bar = 100 µM. TS= Tape-stripping.

500 **Figure 3: Depletion of neutrophils *in vivo* results in decreased *S. aureus* skin**
501 **colonization**

502 **a+b**: WT mice were injected with 100 µg anti-Ly6G antibody. After 24 hours skin was
503 epicutaneously infected with $1x10^8$ *S. aureus*. After 24 hours the percentage of live
504 Ly6G+ CD45+ in the skin and in the blood was analyzed and quantified (quantification
505 for Ly6G+ CD45+ cells in skin is shown) by flow cytometry (**a**) and the number of *S.*
506 *aureus* CFU colonizing the skin was determined (**b**). Horizontal lines represent the
507 mean of each group consisting of 9 mice +/- SEM. **c+d**: The skin of WT and IL-1R1-ko
508 mice was epicutaneously infected with $1x10^8$ *S. aureus*. After 24 hours mice the
509 percentage of live Ly6G+ CD45+ cells in the skin of 3 mice was analyzed by flow
510 cytometry (**c**) and the number of *S. aureus* CFU on the skin surface (wash) as well as
511 in deeper skin tissue (scrape) of 13 mice was determined (**d**). Horizontal lines
512 represent the mean of each group +/- SEM. Two dots represent one mouse (left and
513 right flank). Significant differences to control treatments were analyzed by an unpaired
514 two-tailed t-test (*P<0.05; ****P<0.0001).

515 **Figure 4: *In vitro* co-culture with PMNs specifically increases *S. aureus***
516 **colonization independent of virulence factors and lipopeptides**

517 **a**: Schematic overview of the co-culture experiments. **b**: $1x10^6$ isolated PBMCs or
518 PMNs were co-cultured with differentiated primary human keratinocytes (PHKs) in 0.4
519 µm inserts for 18 hours. Next, PHKs were infected with *S. aureus* (MOI=30) for 1.5
520 hours followed by PHK lysis and analysis of *S. aureus* CFU. Data represent the mean
521 values of one representative experiment with 6 technical replicates +/- SEM. Significant
522 differences to control treatments were analyzed by ordinary one-way ANOVA followed

by Dunnett's multiple comparisons test (****P<0.0001). **c+e**: 1x10^6 isolated PMNs were co-cultured with differentiated PHKs in 0.4 µm inserts for 18 hours. Next, PHKs were infected with *S. epidermidis* (MOI=30) (A) *S. aureus* (MOI=30) WT, *ΔagrΔsae* or *Δlgt* (C) for 1.5 hours followed by PHK lysis and analysis of CFU. Data represent the mean values of one representative experiment with 6 technical replicates +/- SEM. Significant differences to control treatments were analyzed by an unpaired two-tailed t-test (****P<0.0001). **d:** Dorsal skin of WT mice was either tape-stripped or left untreated. Subsequently, skin was epicutaneously infected with 1x10^9 *S. epidermidis*. After 24 hours the number of CFU colonizing the skin was determined. **f**: Dorsal skin of WT/MyD88-ko/TLR2-ko/TLR9-ko mice was tape-stripped. Subsequently, skin was epicutaneously infected with 1x10^8 *S. aureus*. After 24 hours the number of CFU colonizing the tape-stripped skin was determined. Horizontal lines represent the mean of each group +/- SEM. TS= Tape-stripping.

Figure 5: NET formation by neutrophils leads to enhanced *S. aureus* colonization
1x10^6 isolated PMNs were co-cultured with differentiated primary human keratinocytes (PHKs) in 0.4 µm inserts for 18 hours. **a**: 2 hours prior to infection 0.05 mg/mL DNAseI was added to the PMN compartment. Next, PHKs were infected with *S. aureus* (MOI=30) for 1.5 hours followed by PHK lysis and analysis of *S. aureus* CFU. Data represent the mean values normalized to control treatments of three independent experiments with 6 replicates each +/- SEM. Significant differences to control treatments were analyzed by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test (****P<0.0001). **b:** Next, PHKs were infected with *S. aureus* or *S. epidermidis* (MOI=30) for 1.5 hours followed by addition of 5 µM SYTOX™ to the PMN compartment. Control treatments included 500 nM PMA, 0.05 mg/mL DNAseI or 1% Triton-X-100 for 1.5 hours. Samples were transferred in triplicates to a 96-well plate

548 and fluorescence of extracellular DNA was measured. Data were normalized to the
549 Triton X-100 control treatment. **c**: Next, PHKs were infected with *S. aureus* for 1.5
550 hours. PMN supernatant was used for anti-dsDNA ELISA. According to a NET
551 standard, samples are expressed as % of NET standard. Medium only and 0.05 mg/mL
552 DNAseI served as control treatments. **d**: PMNs were seeded onto Poly-L-Lysine
553 coated cover slips with or without PHKs in the co-culture. In the following PMNs were
554 either directly infected or PHKs were infected with *S. aureus* or *S. epidermidis*
555 (MOI=30) for 3.5 hours or treated with 500 nM PMA as a positive control. After washing
556 cells were fixed and stained with indicated antibodies and YOPRO. Representative
557 confocal microscopy images depict citH3 (blue), MPO (red), merge of citH3 and MPO
558 (pink) or YOPRO (green). Scale bar = 10 µm. **e**: Isolated PMNs were treated with 500
559 nM PMA for 4 hours. Subsequently, NETs were purified and added to the lower
560 compartment in a PHK-NET co-culture for 18 hours. Next, PHKs were infected with *S.*
561 *aureus* (MOI=30) for 1.5 hours followed by PHK lysis and analysis of *S. aureus* CFU.
562 Normal PMN co-culture as well as DNAseI treatment served as controls. f: PHKs, were
563 treated with increasing concentrations of isolated NETs or 0.1% Triton X-100 as
564 negative control for 24 hours and subsequently incubated with 4-methylumbelliferyl
565 heptanoate. After 1 hour of incubation, viability was measured and data were
566 normalized to the untreated control. g: genomic DNA from neutrophils was purified and
567 added to the lower compartment in a PHK-gDNA co-culture for 18 hours. Additionally,
568 10 µM GSK484 and 100 µM Cl-Amidine was added to the PMN compartment for 1
569 hour to inhibit NET formation. Next, PHKs were infected with *S. aureus* (MOI=30) for
570 1.5 hours followed by PHK lysis and analysis of *S. aureus* CFU. Normal PMN co-
571 culture as well as DNAseI treatment served as controls. One representative
572 experiment of three independent experiments with 3-6 replicates each +/- SEM is
573 shown. Significant differences to control treatments were analyzed by ordinary one-

574 way ANOVA followed by Sidak's multiple comparisons test (**P<0.01; ***P<0.001;
575 ****P<0.0001).
576
577 **Figure 6: Skin commensals modulate keratinocyte-mediated neutrophil**
578 **recruitment**
579 **a**: PMN migration assay: Isolated PMNs were labeled with 1 µM calcein and were
580 added into a 3 µm insert. The lower chamber contained supernatant of primary human
581 keratinocytes (PHKs) which were previously treated with the indicated bacteria,
582 medium or 10 nM fMLF as a positive control. After 1 hour inserts were removed and
583 samples with migrated neutrophils were transferred to a 96-well plate. The number of
584 migrated neutrophils was analyzed by measuring the calcein fluorescence and
585 correlating values to a neutrophil standard curve. Data represent the mean values of
586 one representative experiment with 3 replicates +/- SEM. Significant differences to
587 control treatments were analyzed by ordinary one-way ANOVA followed by Tukey's
588 multiple comparisons test (*P<0.05; **P<0.01; ***P<0.001). M= Medium; fMLF= N-
589 formyl-met-leu-phe; *S. epi.*= *S. epidermidis*; CM= conditioned medium. **b+c**: The skin
590 of WT mice was pretreated either with PBS or $1x10^9$ *S. epidermidis* for 24 hours. The
591 next day, $1x10^8$ *S. aureus*, $1x10^9$ *S. epidermidis* or PBS were epicutaneously applied.
592 After 24 hours the percentage of neutrophils in the skin of 7 mice was analyzed by flow
593 cytometry (**b**) and 4 mm skin punches were taken and further cultured *in vitro* for 10
594 hours followed by cytokine analysis of the culture supernatant by LEGENDplex™
595 (BioLegend) (**c**). Horizontal lines represent the mean of each group +/- SEM.
596 Significant differences to control treatments were analyzed by ordinary one-way
597 ANOVA followed by Tukey's multiple comparisons test (*P<0.05; **P<0.01;
598 ***P<0.001; ****P<0.0001).

Graphical abstract: Model of *S. aureus* colonization during skin homeostasis and inflammation.

During skin homeostasis (left panel) an alliance of keratinocytes and the skin microbiota collectively prevents *S. aureus* from colonizing the skin by reducing the recruitment of neutrophils. However, upon skin colonization with *S. aureus* or induction of skin inflammation (right panel), neutrophils are recruited. Soluble factors secreted by *S. aureus* infected keratinocytes induce PMNs to release NETs, which influences PHKs in a yet unknown way that favors colonization of *S. aureus*.

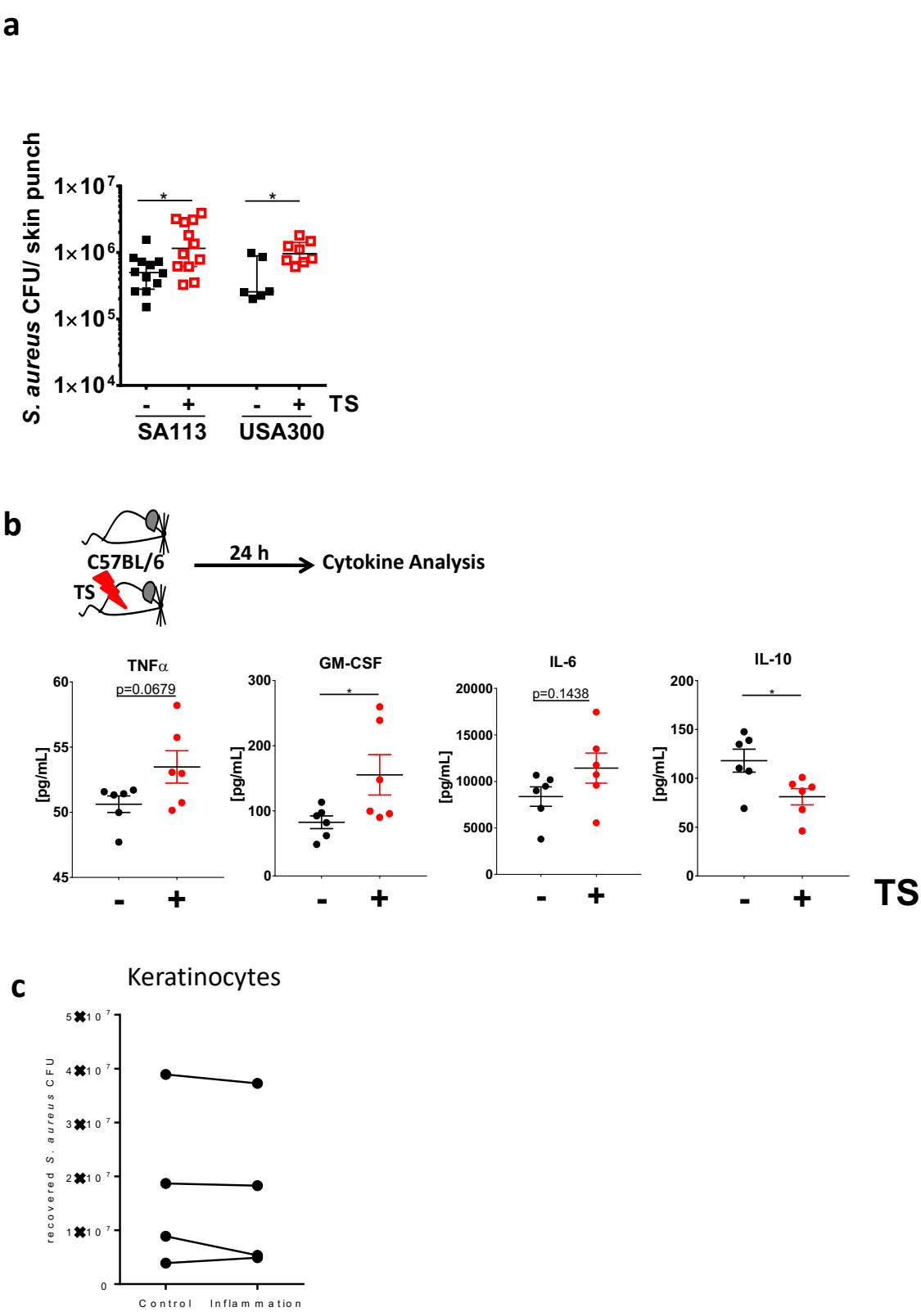

a
S. aureus CFU/ skin punch
1×10^7
1×10^6
1×10^5
1×10^4
*
*
- + - + TS
SA113 USA300
b
C57BL/6
TS
24 h
Cytokine Analysis
TNFα
p=0.0679
GM-CSF
*
IL-6
p=0.1438
IL-10
*
[pg/mL]
60
55
50
45
300
200
100
0
20000
15000
10000
5000
200
150
100
50
- + - + - + - + TS
c
Keratinocytes
recovered S. aureus CFU
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
Control
Inflammation

Figure 1

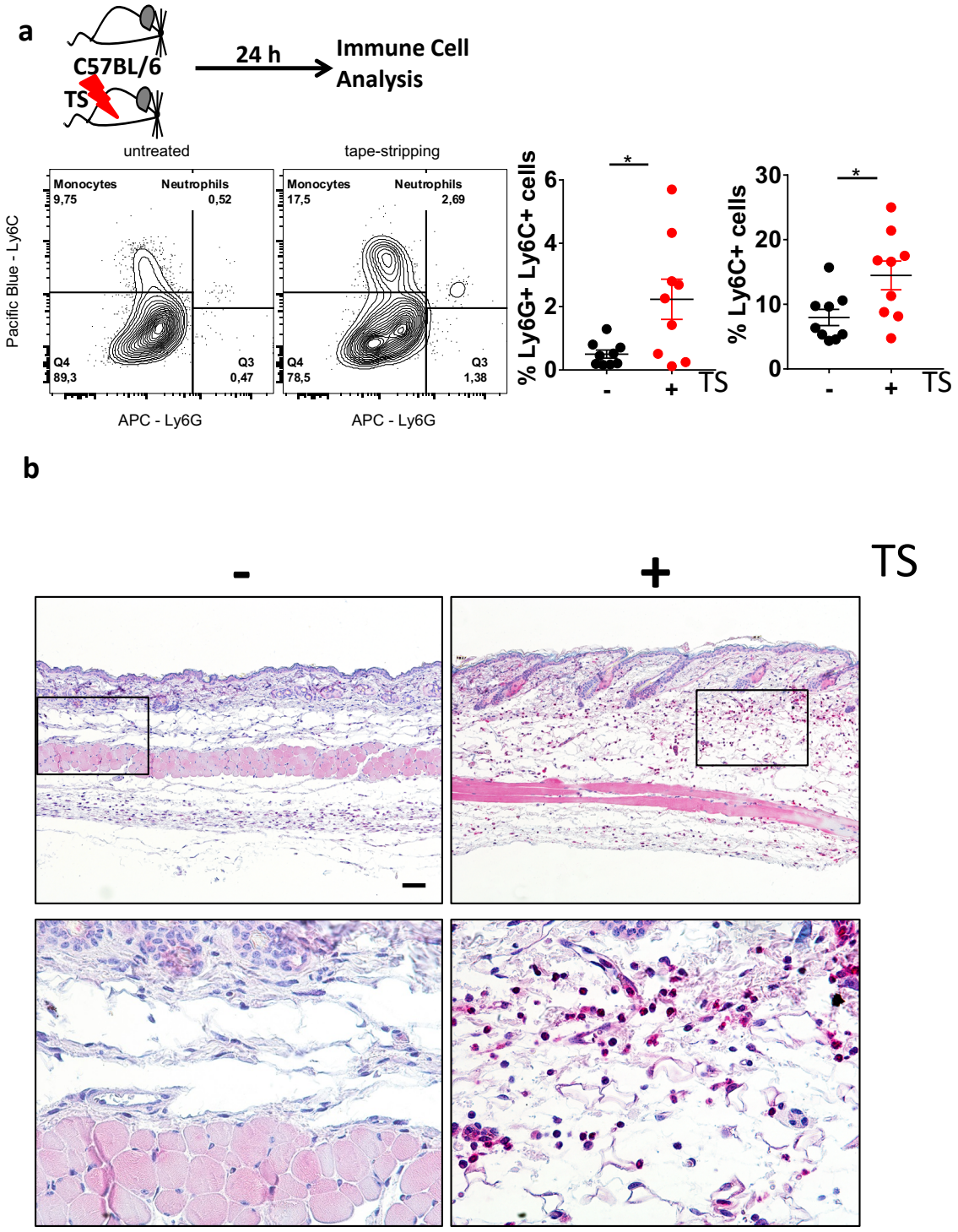

a
C57BL/6
TS
24 h
Immune Cell Analysis
untreated
tape-stripping
Monocytes
9,75
Neutrophils
0,52
Q4
89,3
Q3
0,47
Monocytes
17,5
Neutrophils
2,69
Q4
78,5
Q3
1,38
Pacific Blue - Ly6C
APC - Ly6G
APC - Ly6G
% Ly6G+ Ly6C+ cells
% Ly6C+ cells
*
*
-
+
TS
b
-
+
TS

Figure 2

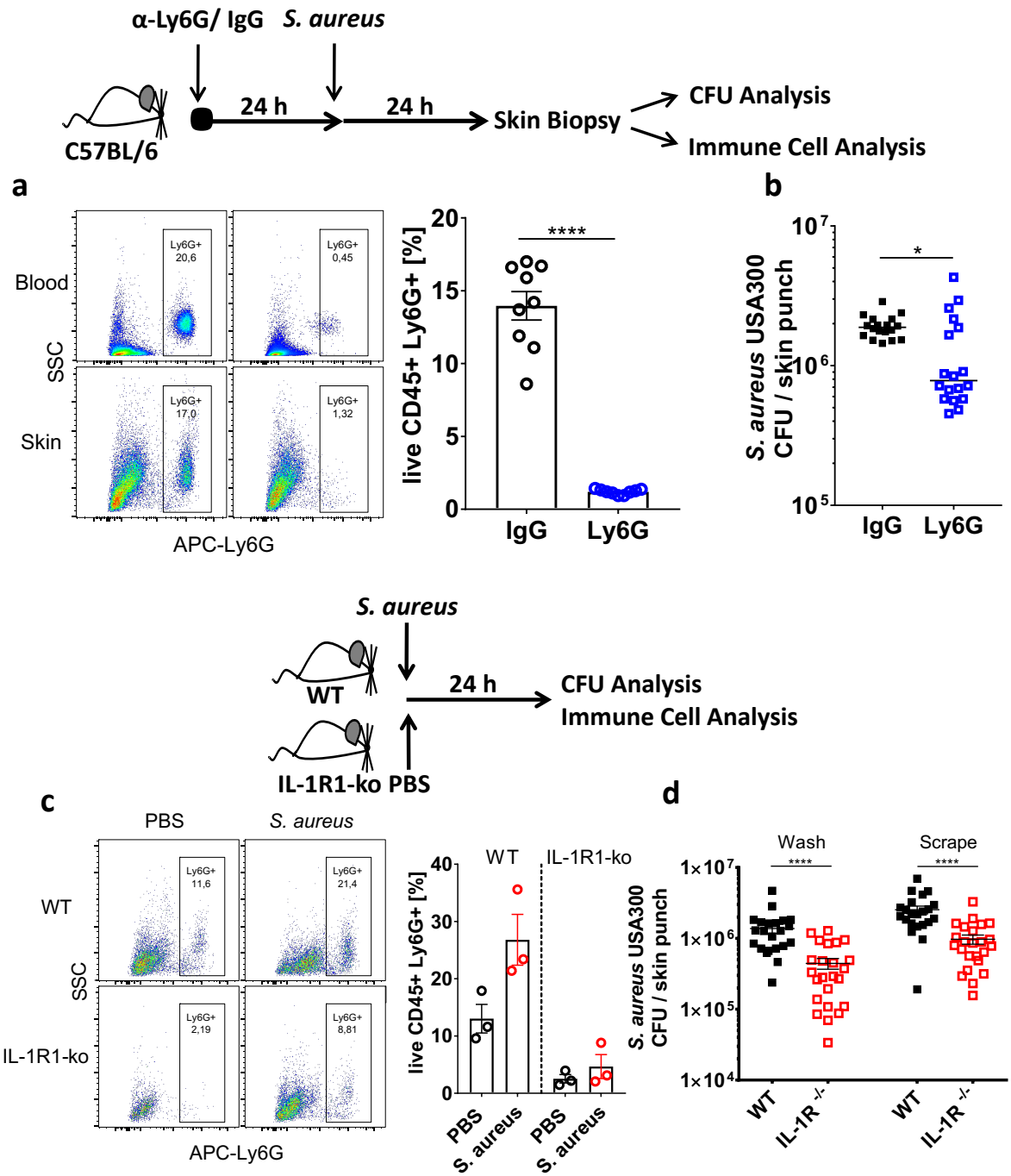

α-Ly6G/ IgG
S. aureus
24 h
24 h
Skin Biopsy
CFU Analysis
Immune Cell Analysis
C57BL/6
a
Blood
Skin
SSC
APC-Ly6G
Ly6G+ 20,6
Ly6G+ 0,45
Ly6G+ 17,0
Ly6G+ 1,32
live CD45+ Ly6G+ [%]
****
IgG
Ly6G
b
S. aureus USA300 CFU / skin punch
*
IgG
Ly6G
S. aureus
WT
24 h
CFU Analysis
Immune Cell Analysis
IL-1R1-ko
PBS
c
PBS
S. aureus
WT
IL-1R1-ko
SSC
APC-Ly6G
Ly6G+ 11,6
Ly6G+ 21,4
Ly6G+ 2,19
Ly6G+ 8,81
live CD45+ Ly6G+ [%]
WT
IL-1R1-ko
PBS
S. aureus
d
Wash
Scrape
****
S. aureus USA300 CFU / skin punch
WT
IL-1R -/-

Figure 3

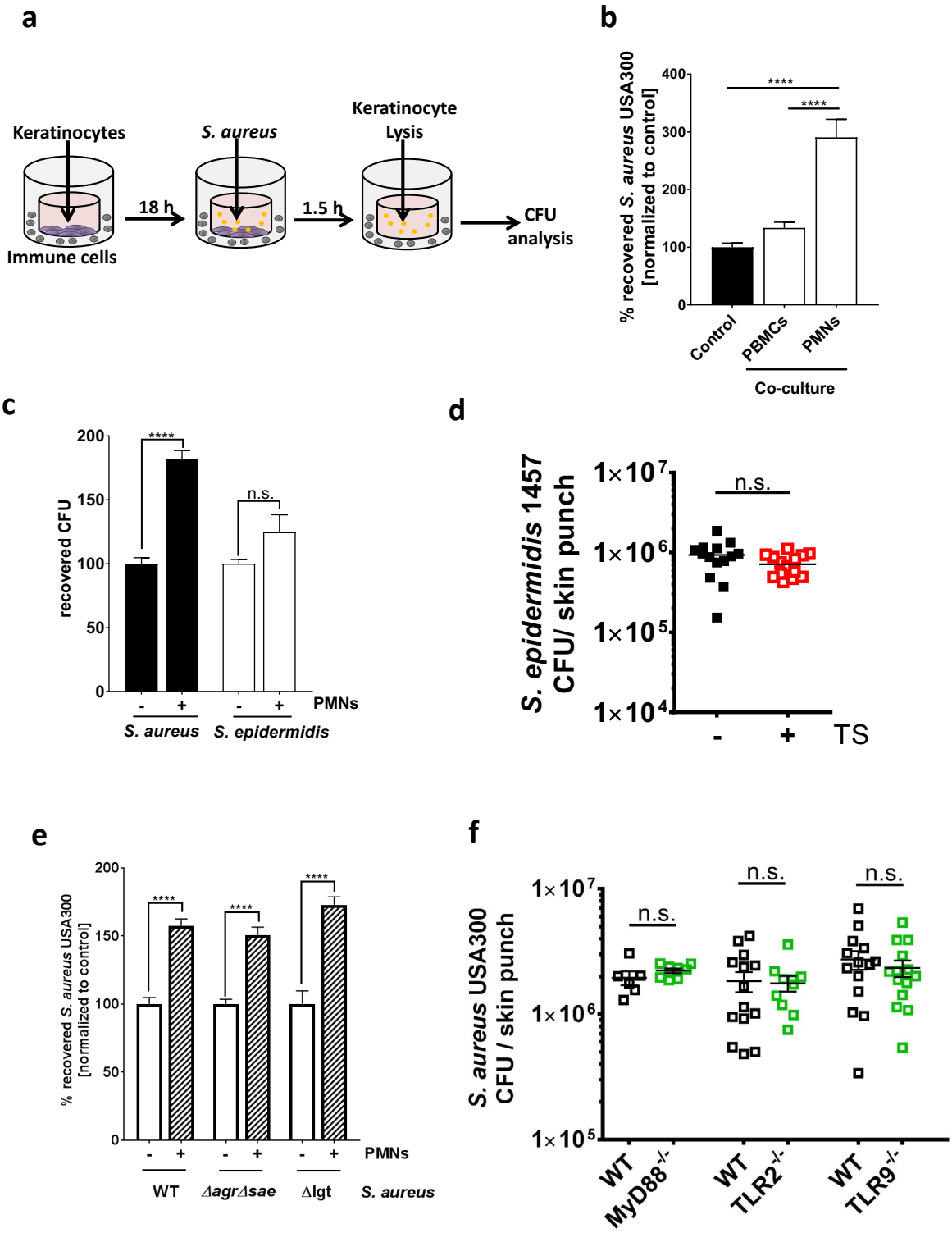

a
Keratinocytes
S. aureus
Keratinocyte Lysis
18 h
1.5 h
CFU analysis
Immune cells
b
% recovered S. aureus USA300 [normalized to control]
****
****
Control
PBMCs
PMNs
Co-culture
c
recovered CFU
****
n.s.
PMNs
S. aureus
S. epidermidis
d
S. epidermidis 1457 CFU/ skin punch
n.s.
TS
e
% recovered S. aureus USA300 [normalized to control]
PMNs
WT
ΔagrΔsae
Δlgt
S. aureus
f
S. aureus USA300 CFU / skin punch
n.s.
n.s.
n.s.
WT
MyD88-/-
WT
TLR2-/-
WT
TLR9-/-

Figure 4

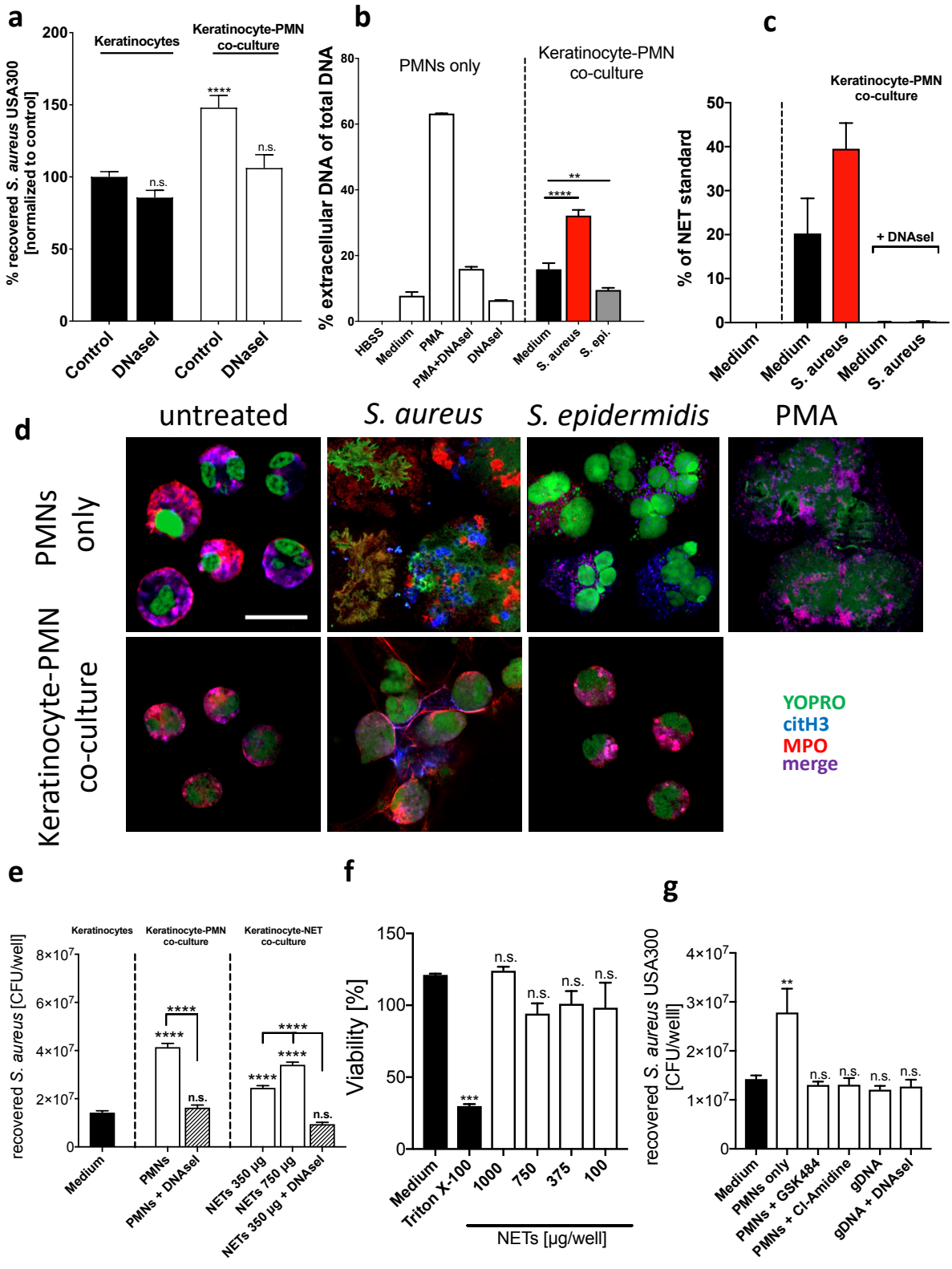

a
% recovered S. aureus USA300 [normalized to control]
Keratinocytes
Keratinocyte-PMN co-culture
Control
DNaseI
b
% extracellular DNA of total DNA
PMNs only
Keratinocyte-PMN co-culture
HBSS
Medium
PMA
PMA+DNAseI
DNAseI
S. aureus
S. epi.
c
% of NET standard
Keratinocyte-PMN co-culture
+ DNAseI
d
untreated
S. aureus
S. epidermidis
PMA
PMNs only
Keratinocyte-PMN co-culture
YOPRO
citH3
MPO
merge
e
recovered S. aureus [CFU/well]
Keratinocytes
Keratinocyte-PMN co-culture
Keratinocyte-NET co-culture
PMNs
PMNs + DNAseI
NETs 350 µg
NETs 750 µg
NETs 350 µg + DNAseI
f
Viability [%]
Triton X-100
NETs [µg/well]
g
recovered S. aureus USA300 [CFU/well]
PMNs only
PMNs + GSK484
PMNs + Cl-Amidine
gDNA
gDNA + DNAseI

Figure 5

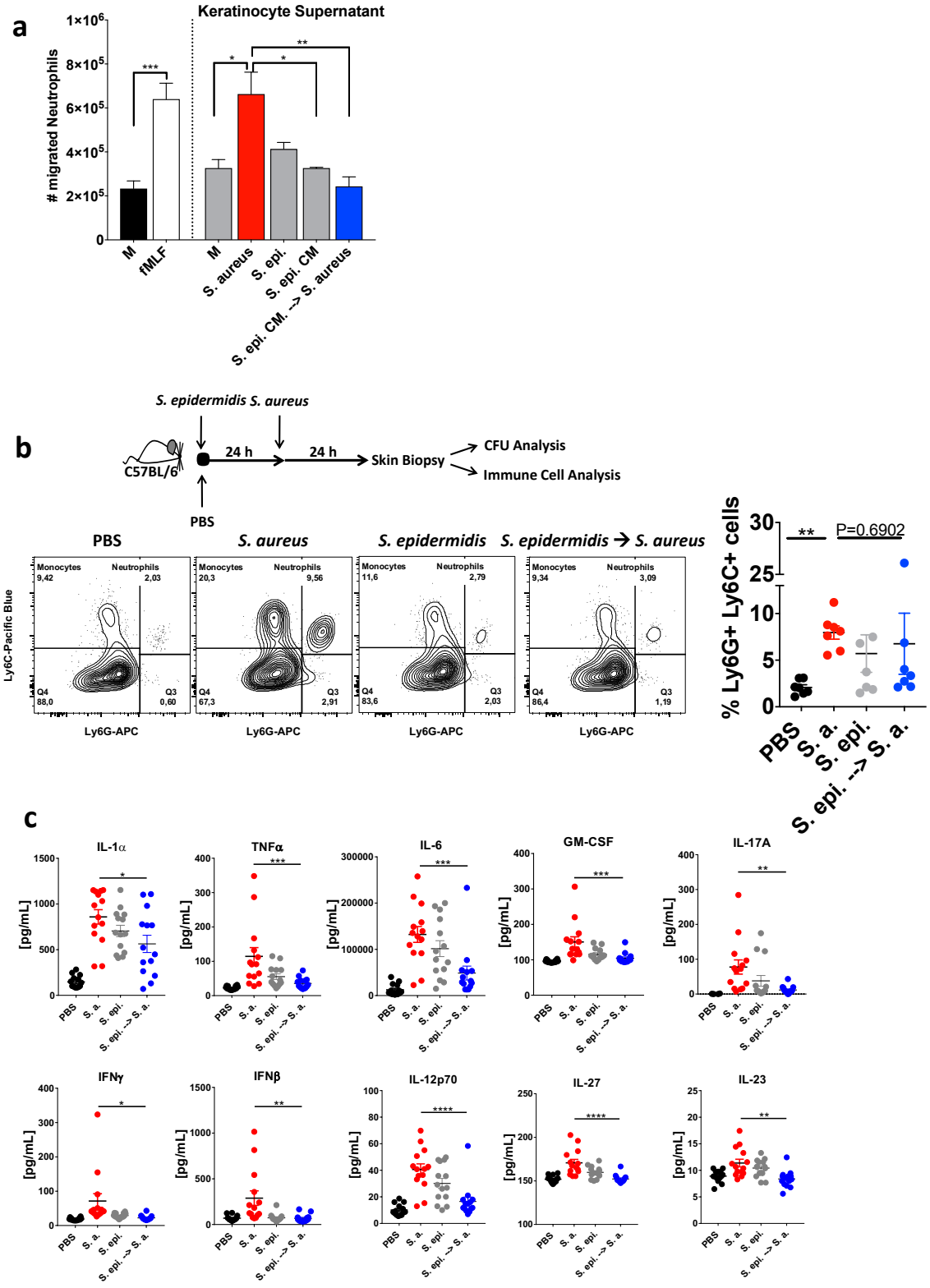
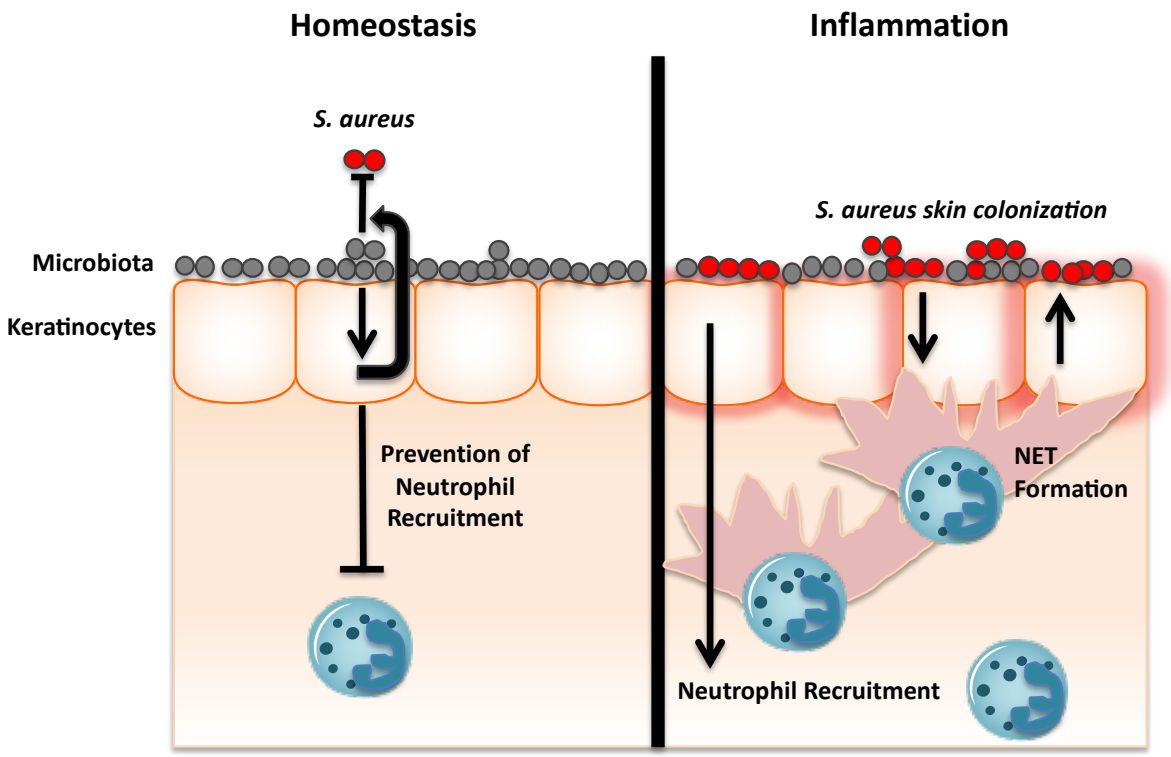

a
Keratinocyte Supernatant
migrated Neutrophils
M
fMLF
M
S. aureus
S. epi.
S. epi. CM
S. epi. CM. --> S. aureus
b
S. epidermidis S. aureus
C57BL/6
24 h
24 h
Skin Biopsy
CFU Analysis
Immune Cell Analysis
PBS
PBS
S. aureus
S. epidermidis
S. epidermidis → S. aureus
Ly6C-Pacific Blue
Ly6G-APC
% Ly6G+ Ly6C+ cells
P=0.6902
c
IL-1α
TNFα
IL-6
GM-CSF
IL-17A
IFNγ
IFNβ
IL-12p70
IL-27
IL-23
[pg/mL]

Figure 6



Graphical Abstract

Supplementary Figure Legends

Figure S1: Skin inflammation increases *S. aureus* colonization *in vivo*

A: Dorsal skin of WT mice was either tape-stripped or left untreated. After 24 hours 4 mm skin punches were taken and further cultured *in vitro* for 10 hours followed by cytokine analysis of the culture supernatant by LEGENDplex™ (BioLegend) and MIP-2 ELISA (R&D Systems). Shown are the mean concentrations of the indicated cytokines in the supernatant of the skin culture of one skin punch from 6 mice +/- SEM. TS= Tape stripping. **B**: PHKs were treated with a pro-inflammatory cytokine cocktail (10 ng/mL IL-1α, TNFα, Oncostatin M, IL-22 and IL-17A) for 3 hours. Subsequently, cells were lysed, total RNA was isolated and expression of indicated cytokines was analyzed by qRT-PCR.

Figure S2: Gating strategy and quantification for immune cell analysis in mouse skin

A+B: Gating strategy and quantification for live CD45+ cells. **C-F**: Gating for the different immune cell subsets pre-gated on live CD45+ cells: CD11b+ and CD11c+ cells (**C**); F4/80+ CD11b+ macrophages (**D**); CD19+ B cells (**E**); CD3+ T cells, NK1.1+ CD3+ NKT cells and NK1.1+ NK cells (**F**). Shown is the mean percentage of indicated immune cells in mouse skin of 9 C5BL/6 WT mice +/- SEM. One dot represents one mouse.

Figure S3: *In vitro* co-culture with PMNs increases *S. aureus* colonization of keratinocytes

A: $1x10^6$ isolated immune cells were co-cultured (right) or not (left) with differentiated PHKs in 0.4 µm inserts for 18 hours. Next, PHKs were infected with *S. aureus* (MOI=30) for 1.5 hours in the presence (left) or absence (right) of immune cells followed by PHK lysis and analysis of *S. aureus* CFU. Data represent the mean values normalized to control treatments of three independent experiments with 6 replicates each +/- SEM. **B**: Supernatant from an 18 hours culture of $1x10^6$ isolated PMNs was incubated with differentiated PHKs for 18 hours. Next, PHKs were infected with *S. aureus* (MOI=30) for 1.5 hours followed by PHK lysis and analysis of *S. aureus* CFU. Data represent the mean values normalized to control treatments of three independent experiments with 6 replicates each +/- SEM.

Figure S4: DNAseI treatment reduces *S. aureus* colonization in PMN-PHK co-culture

$1x10^6$ isolated PMNs were co-cultured with differentiated PHKs in 0.4 µm inserts for 18 h. **a:** 2 hours prior to infection 0.05 mg/mL DNAseI, 500 nM PMA, 1x cOmplete™ protease inhibitor, 500 ng/mL ionomycin, 10 nM fMLF were added to the PMN compartment. Next, PHKs were infected with *S. aureus* (MOI=30) for 1.5 hours followed by PHK lysis and analysis of *S. aureus* CFU. Data represent the mean values normalized to control treatments of three independent experiments with 6 replicates each +/- SEM. Significant differences to control treatments were analyzed by ordinary one-way ANOVA followed by Dunnett's multiple comparisons test (***$P<0.001$). b: 1 hour prior to 500 nM PMA treatment, the PMN compartment was treated with 10 µM GSK484, 100 µM Cl-Amidine or 0.05 mg/mL DNAseI. After 4 hours, 5 µM SYTOX™ was added to the PMN compartment. Control treatments included medium only, 500 nM PMA only, 0.05 mg/mL DNAseI or 1% Triton-X-100. Samples were transferred in triplicates to a 96-well plate and fluorescence of extracellular DNA was measured. Data were normalized to the Triton X-100 control treatment.

Figure S5: Skin commensals modulate *S. aureus*-induced pro-inflammatory cytokine release

The skin of WT mice was pretreated either with PBS or $1x10^9$ *S. epidermidis*. The next day, $1x10^8$ *S. aureus*, $1x10^9$ *S. epidermidis* or PBS were epicutaneously applied. After 24 hours 4 mm skin punches were taken and further cultured *in vitro* for 10 hours followed by cytokine analysis of the culture supernatant by LEGENDplex™ (BioLegend). Horizontal lines represent the mean of each group +/- SEM.

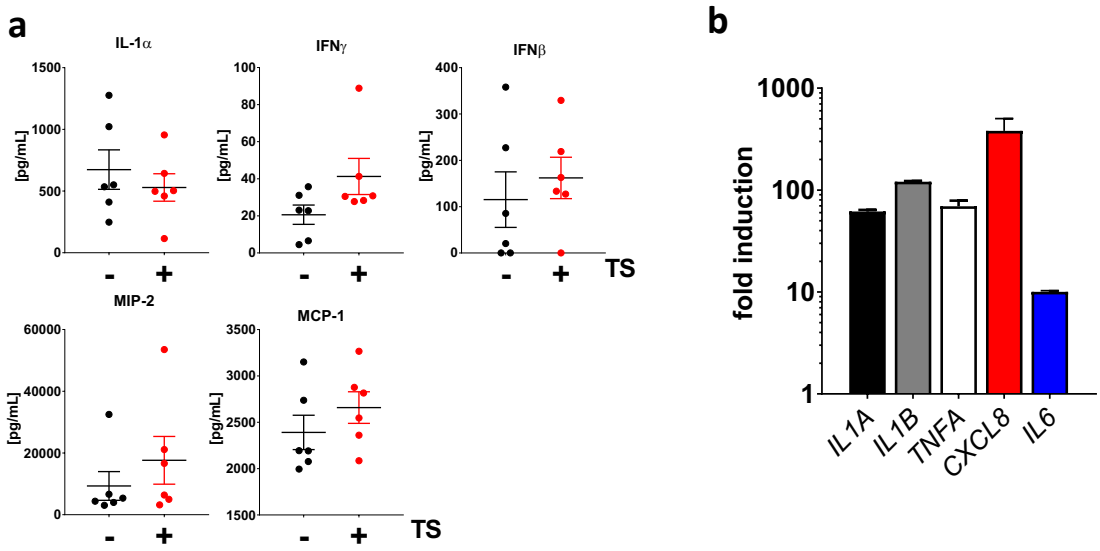


Figure S1: Skin inflammation increases *S. aureus* skin colonization *in vivo*

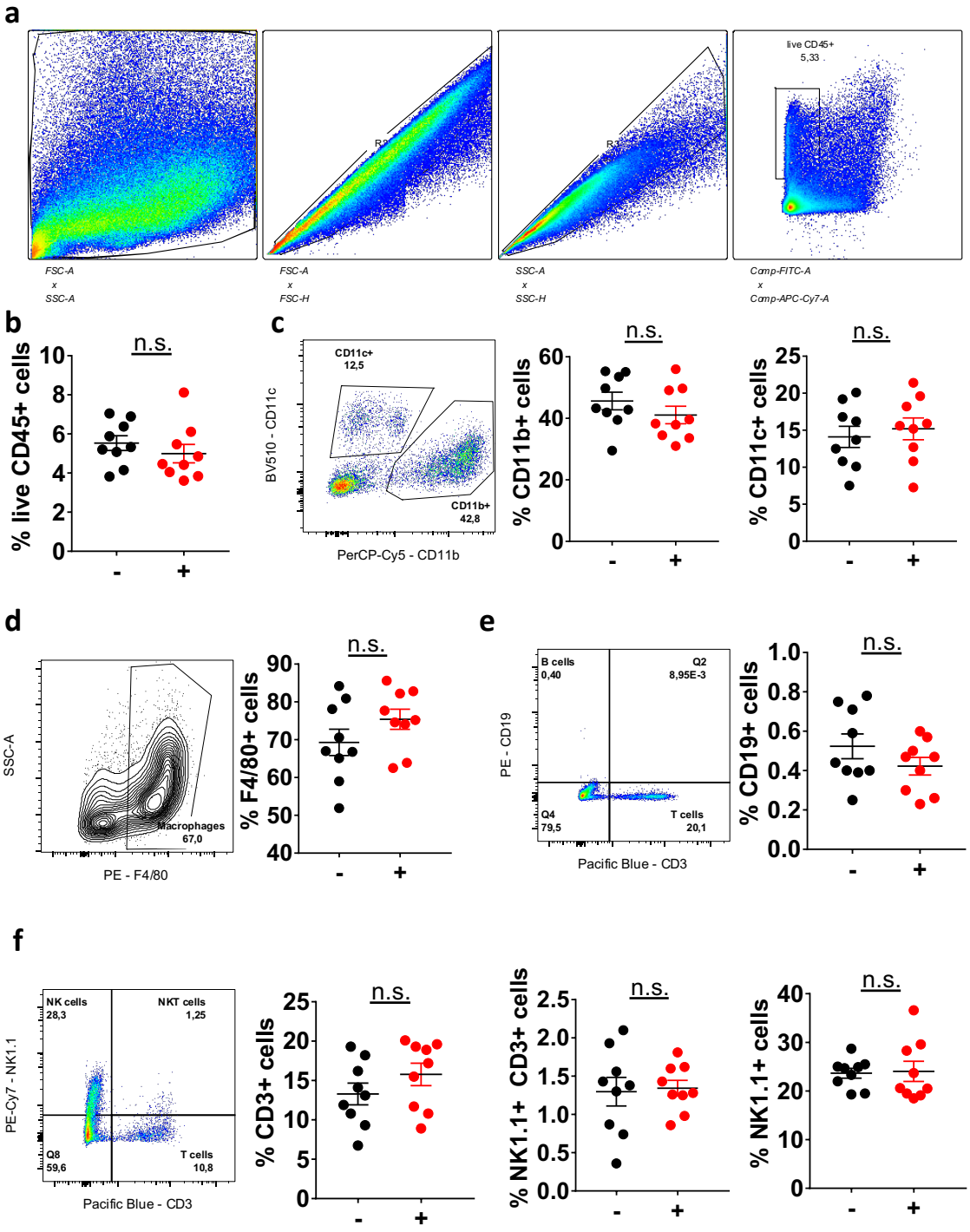


Figure S2: Gating strategy and quantification for immune cell analysis in mouse skin

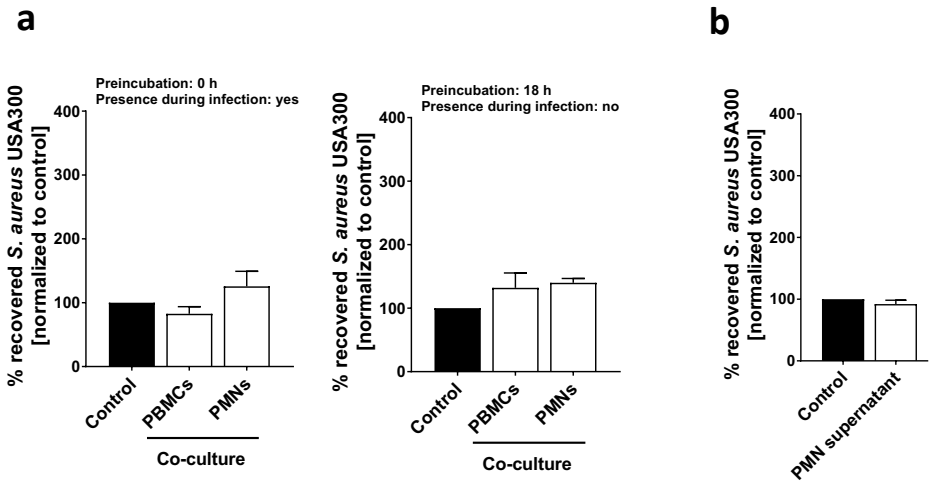


Figure S3: *In vitro* co-culture with PMNs increases *S. aureus* colonization of keratinocytes

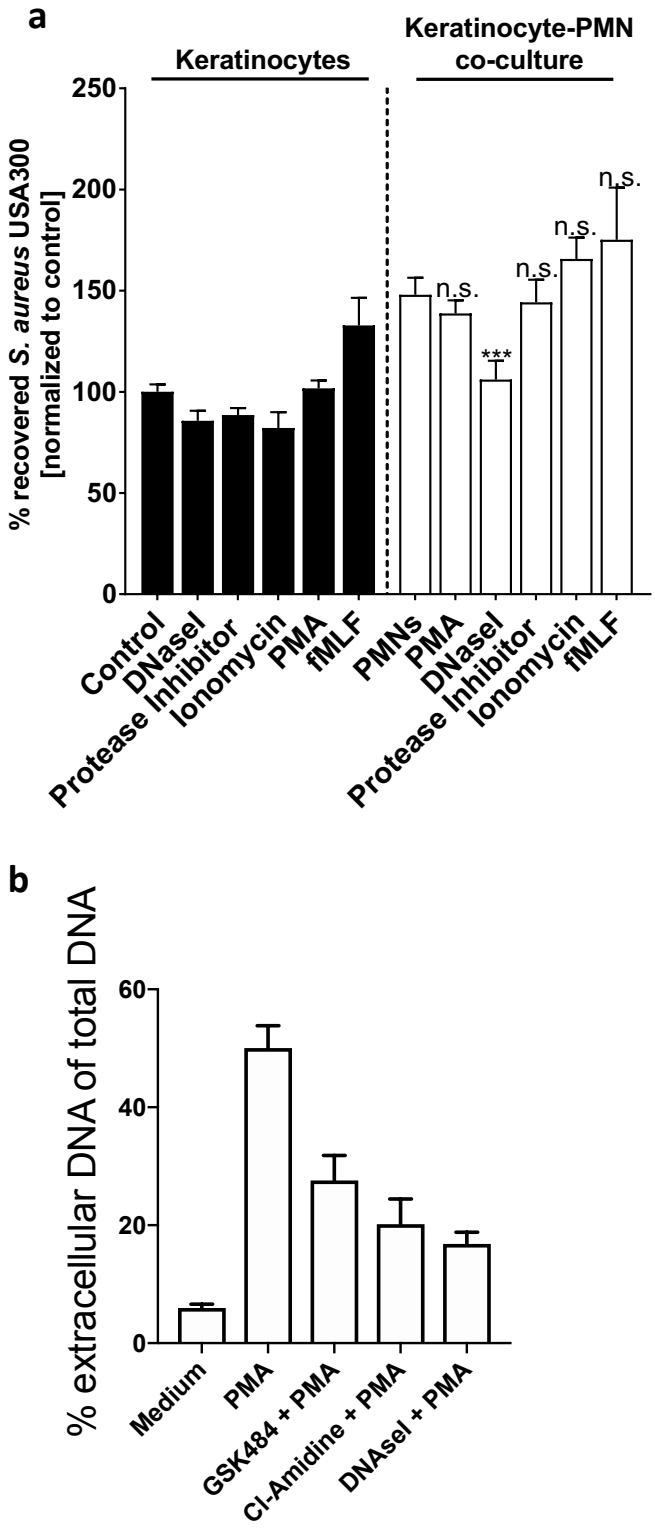


Figure S4: DNAseI treatment reduces *S. aureus* colonization in PMN-PHK co-culture

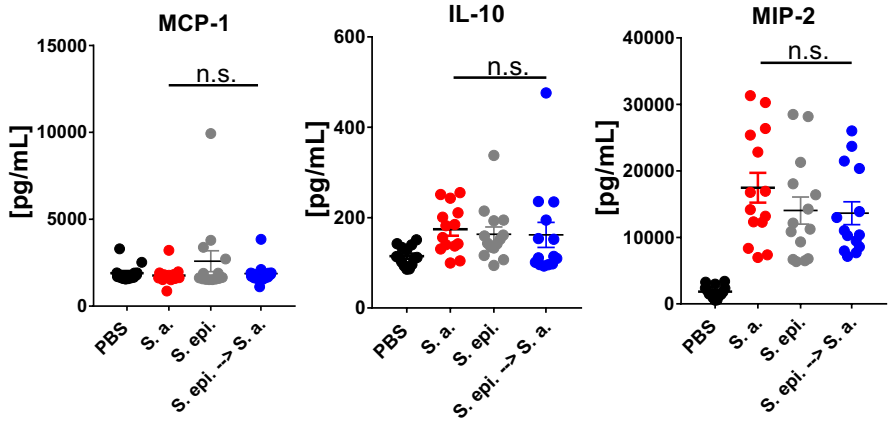


Figure S5: Skin commensals modulate *S. aureus*-induced pro-inflammatory cytokine release

Supplementary Materials and Methods

Bacterial strains and culture conditions

The Staphylococci used in this study were *S. aureus* USA300 LAC, *S. epidermidis* 1457 as well as the *S. aureus* USA300 mutants Δ*agr*Δ*sae* (Munzenmayer et al., 2016) and Δ*lgt*. The insertional inactivation of *lgt* was transferred from strain SA113 lgt::ermB (Stoll et al., 2005) into strain USA300 via Φ11-mediated transduction. Staphylococci were aerobically grown in tryptic soy broth (TSB) at 37 °C and orbital shaking. All assays were performed with logarithmically growing (OD=0.5) bacteria. *S. epidermidis* conditioned medium (CM) was generated by culturing *S. epidermidis* 1457 in keratinocyte CnT base medium (CELLnTEC) for 18 hours at 37 °C and orbital shaking followed by centrifugation and filter-sterilization.

In vivo skin colonization model

Animal studies were performed with 6-8-week-old female C57BL/6 WT/IL-1R1/MyD88-ko/TLR2-ko/TLR9-ko mice. Mouse skin was shaved 3 days prior to experiments, allowing potential micro wounds to heal and skin to recover from shaving. Tape-stripping was conducted by repeatedly (7x) pulling off tape from the shaved dorsal skin of mice prior to colonization as previously described (Burian et al., 2017). To analyze *S. aureus* skin colonization, 15 µL of a bacterial suspension containing $1x10^8$ *S. aureus* were epicutaneously applied for 24 hours on the shaved dorsal skin of mice by using Finn Chambers (Smart Practice). After 24 hours mice were euthanized and 4 mm skin punches were used for *S. aureus* CFU analysis as previously described (Bitschar et al., 2019; Burian et al., 2017; Zipperer et al., 2016). Here, washing of the skin punch in PBS yielded the "wash" fraction and further scalpel-mediated mechanical disruption of the skin punch yielded the "scrape" fraction. For *S. epidermidis* pretreatment, 15 µL containing $1x10^9$ *S. epidermidis* or PBS as a control were epicutaneously applied using

Finn Chambers for 24 hours on the shaved dorsal skin of mice as previously described. The next day, Finn Chambers were removed and 15 µL of a bacterial suspension containing $1x10^8$ *S. aureus* were epicutaneously applied.

Neutrophil depletion in vivo

For neutrophil depletion 100 µg of anti-Ly6G or IgG control antibody (BioXCell, clone 1A8 and clone 2A3) were injected intraperitoneally 24 hours prior to experiments. Efficient depletion was monitored by flow cytometry.

Mouse immune cell isolation and staining procedure

To prepare single-cell suspensions, relevant dorsal skin area was transferred to PBS + 2% FBS (Biochrom/ Merck Millipore). Subcutaneous fat was removed using a razor blade and skin tissue was transferred into a 2 mL reaction tube containing digestion solution (0.05 mg/mL DNaseI (Roche) and 0.25 mg/mL Liberase (Roche) in RPMI-1640 Medium (Gibco/ life technologies)). After scissor-mediated tissue disintegration digestion was performed for 1 hour at 37 °C and stopped by addition of 100 µL of FBS (Biochrom/ Merck Millipore). Single cells were separated by using an 80 µm cell strainer (Greiner Bio-One). After washing in PBS + 2% FBS single cell suspensions were FC-blocked with TruStain fcX™ anti CD16/32 (BioLegend) for 10 min on ice and subsequently surface-stained with the following monoclonal antibodies: CD45.2 (BioLegend clone 104), F4/80 (BioLegend, clone BM8), CD11b (BioLegend, clone M1/70), CD11c (BioLegend, clone N418), Ly6G (BioLegend, clone 1A8), Ly6C (BioLegend, clone HK1.4), CD19 (BioLegend, clone 6D5), CD3 (BioLegend, clone 17A2), NK1.1 (BioLegend, clone PK136). Fixable viability dye eFluor520 (eBioscience™) was used to exclude dead cells. All samples were acquired using a BD LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo (Treestar).

Cell culture

Primary human keratinocytes (PHKs) were isolated from human foreskin after routine circumcision from the Loretto Clinic in Tübingen as previously described (Burian et al., 2017; Nguyen et al., 2017; Whiteley et al., 2017). PHKs were cultured in collagen-coated tissue flasks (Corning, BioCoat™) in epidermal keratinocyte medium (CELLnTEC) at 37 °C, 5% CO_2 as described previously (Burian et al., 2017). 24 hours prior to experiments keratinocytes were differentiated with 1.7 mM $CaCl_2$ in epidermal keratinocyte base medium (CELLnTEC).

Peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs) were cultured in RPMI 1640 (Gibco/ life technologies) containing 10% FBS (Biochrom/ Merck Millipore). For co-culture experiments PBMCs and PMNs were cultured in epidermal keratinocyte base medium (CELLnTEC) containing 1.7 mM CaCl2.

Adhesion and invasion assay

Adhesion and invasion assays were performed as previously described (Burian et al., 2017). Briefly, differentiated PHKs were incubated with pro-inflammatory cytokines (10 ng/mL IL-1α, TNFα, Oncostatin M, IL-22 and IL-17A; PeproTech) or medium as a control for 3 or 18 hours. Subsequently, PHK supernatant was removed, PHKs were washed twice with Hank's Balanced Salt Solution (HBSS) (Sigma) and fresh keratinocyte base medium containing 1.7 mM $CaCl_2$ was added. Subsequently, PHKs were infected with *S. aureus* (MOI=30; OD=0.5) for 1.5 hours. After 2 wash steps with HBSS, PHKs were lysed and serial dilutions of the lysate were plated onto blood agar plates. After overnight incubation at 37 °C CFU were counted.

PBMC and PMN isolation from healthy human blood

Human PBMCs and PMNs were isolated from the peripheral blood of healthy donors by Ficoll-Histopaque (Biochrom) gradient centrifugation. PBMCs were washed once in

PBS and adjusted to a cell number of 2×10^6 per mL in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco/ life technologies) containing 10% FBS (Biochrom/ Merck Millipore). For PMN isolation red blood cells were lysed twice in a hypotonic lysis buffer (0.154 M ammonium chloride, 10 mM potassium bicarbonate, 0.1 mM EDTA, pH 7.4) by rolling at 4 °C for 20 and 10 min. Subsequently, PMNs were adjusted to a cell number of 2×10^6 per mL in RPMI 1640 medium containing 10% FBS.

During the establishment of the co-culture model isolated immune cells where extensively characterized: Ficoll-isolated PBMCs and PMNs exhibited a donor-independent average viability above 99% for the former and above 93% for the latter. Additionally, monocyte contamination (CD14+) of purified PMNs as well as percentages of CD3+, CD3+ CD4+ and CD3+ CD8+ PBMCs where quantified. Purity of PMNs (CD66b+) was always above 98%. Moreover, PMN and PBMC viability in keratinocyte medium was tested and showed no decrease in viability when immune cells were cultured in keratinocytes medium (data not shown).

Composition of PBMCs and purity of PMNs was analyzed by flow cytometry. Surface staining was carried out for 20 min on ice using CD66b (BioLegend, clone G10F5), CD14 (BioLegend clone M5E2), CD62L (BioLegend, clone DREG-56), CD3 (eBioscience™, clone OKT3), CD4 (eBioscience™, clone RPA-T4) and CD8a (eBioscience™, clone OKT8). Fixable viability dye eFluor520 (eBioscience™) was used to exclude dead cells. All samples were acquired using a BD LSRII flow cytometer (BD Biosciences) and analyzed with FlowJo (Treestar).

In vitro co-culture model

For keratinocyte and immune cell co-culture keratinocytes were seeded into collagen-coated 24-well 0.4 µm cell culture inserts (Merck/Millipore). 24 hours prior to the end of the experiment keratinocytes were differentiated with 1.7 mM $CaCl_2$ in epidermal

keratinocyte base medium (CELLnTEC). Isolated immune cells were resuspended in 300 µL epidermal keratinocyte base medium (CELLnTEC) containing 1.7 mM $CaCl_2$ and added to the bottom of the well in which the inserts are placed. Immediately before infection fresh medium was added to keratinocytes and adhesion and invasion assay of *S. aureus* on keratinocytes was performed as described above, but in the presence or in the absence of immune cells as control in the bottom chamber. Normally, pre-incubation time of immune cells and keratinocytes was 18 hours. However, experiments without pre-incubation were performed additionally where indicated only. Moreover, supernatant of PMNs cultured for 18 hours was used instead of living PMNs where indicated. To identify the substance class, 0.05 mg/mL DNAseI (Roche), 500 nM PMA (Sigma), 1x cOmplete™ protease inhibitor (Roche), 500 ng/mL ionomycin (Sigma) or 10 nM fMLF (Sigma) were added to the PMN compartment 2 hours prior to *S. aureus* infection of PHKs. For PAD4 inhibition a final concentration of 10 µM GSK484 (Merck Millipore) or 100 µM Cl-Amidine (Merck Millipore) was added to the PMN compartment 1 hour before *S. aureus* infection of PHKs. To exclude donor-dependent variations all experiments were repeatedly performed with PMNs and PBMCs from different donors.

RNA isolation and cDNA synthesis

PHKs were washed once with PBS followed by addition of RNA lysis buffer directly into the well. Total RNA was extracted using the Nucleospin RNA Kit (MACHEREY-NAGEL) according to the manufacturer's protocol. cDNA was synthesized using the Reverse-Transkriptase Kit (Thermo Fisher Scientific) with 2 µg of RNA, 4 µL of 5x RT buffer, 0.5 µL Maxima reverse transcriptase (200 U/mL), 1 µL of random hexamer primer (100 µM), dNTP (10 mM) and RNAse-free water to a total volume of 20 µL. After pre-incubation of RNA with water for 10 min at 70°C, master mix was added and

incubated for 10 min at 25 °C, followed by 45 min at 50°C and a final heat inactivation step for 5 min at 85°C.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

qRT-PCR was performed in 10 µL reaction volume with SYBR™ Green PCR Master Mix (Thermo Fisher Scientific) according to manufacturer's instructions using a LightCycler 96 (Roche Life Science). The initial denaturation step was at 95°C for 5 min, followed by 40 cycles with 10 sec each for the denaturation step at 95°C, the annealing at individual temperature and the elongation at 72 °C. Primer sequences and respective annealing temperatures were: ***IL1A*** fw: GGTTGAGTTTAAGCCAATCCA rv: TGCTGACCTAGGCTTGATGA (58 °C), ***IL1B*** fw: CTGTCCTGCGTGTTGAAAGA rv: TTGGGTAATTTTTGGGATCTACA (58 °C), ***TNFA*** fw: CCCAGGCAGTCAGATCATCTTC rv: AGCTGCCCCTCAGCTTGA (60 °C), ***CXCL8*** fw: AGACAGCAGAGCACACAAGC rv: ATGGTTCCTTCCGGTGGT (60 °C), ***IL6*** fw: CAGGAGCCCAGCTATGAACT rv: GAAGGCAGCAGGCAACAC (59 °C).

LEGENDplex™ multiplex cytokine analysis

For cytokine analysis from human cell cultures, 10 µL of supernatant was used for cytokine analysis via the LEGENDplex™ human inflammation panel (BioLegend).

For cytokine analysis from mouse skin, 4 mm skin punches from the relevant area were cultured in an airlift system where only the dermis had access to medium for 10 hours. Skin punches were cultured in RPMI containing 1% FBS (Biochrom/ Merck Millipore), 1% penicillin and streptomycin (Gibco/ life technologies) and 0.25 µg/mL amphotericin B (CELLnTEC). 10 µL of a 3-fold dilution of culture supernatant was used for cytokine analysis via the LEGENDplex™ mouse inflammation panel (BioLegend). Samples were acquired in duplicates using a BD LSRII flow cytometer (BD Biosciences) and LEGENDplex™ Software (BioLegend).

PMN Boyden chamber migration assay

For the PMN migration assay differentiated PHKs were treated with indicated bacteria (multiplicity of infection = 30) for 1.5 hours. For *S. epidermidis* CM pretreatment PHKs were pretreated with *S. epidermidis* CM for 18 hours followed by medium change and subsequent infection with *S. aureus* for 1.5 hours. After 1.5 hours of infection, PHK supernatant was removed, filter-sterilized and used as a stimulus in the PMN Boyden chamber migration assay: Therefore, freshly-isolated PMNs were labelled with 1 µM Calcein (eBioscience™) for 30 min. After washing PMNs were added into a 3 µm insert (Sarstedt) and PMN stimulus was added to the lower compartment. Here, medium or 10 nM fMLF served as controls. After 1 hour, inserts were removed and migrated PMNs in the lower compartment were lysed using 1% Triton X-100. Lysates were transferred in triplicates to a 96-well plate and the fluorescence (λ_{ex}: 488 nm λ_{em}: 523 nm) was measured using a Fluoroskan II (Labsystems). 10 nM fMLF served as a positive control. To calculate absolute numbers of migrated PMNs a standard curve was always included.

Neutrophil extracellular traps quantification assay (SYTOX™)

PHKs and PMNs were co-cultured and infected as described above. After 3 hours, the supernatant of the PMN compartment was transferred to a 96-well plate. 5 µM of SYTOX™ (Thermo Fisher) was added and the fluorescence (λ_{ex}: 488 nm λ_{em}: 523 nm) was measured using a Fluoroskan II (Labsystems). 10% Triton X-100 was used as a total DNA content control, 500 nM PMA was used as a positive control and 0.05 mg/mL DNAseI was used as a negative control.

ELISA

For MIP-2 analysis, 100 µL of a 5-fold dilution from skin culture supernatant was used and analyzed via ELISA (R & D Systems) according to manufacturer's instruction.

The protocol for the dsDNA ELISA was adapted from (Sil et al., 2017). For the anti dsDNA ELISA a NET standard curve was prepared by stimulating $2*10^6$ neutrophils with 500 nM PMA for 4 hours at 37 °C. Subsequently, 2 U/mL DNaseI were added for 15 minutes followed by addition of 2.5 mM EGTA to stop DNaseI digestion. After centrifugation the supernatant was diluted 1:5 in PBS containing 0.5 M EGTA and a two-fold diluted standard curve was prepared.

For the ELISA, PMN supernatant from the co-culture was collected before and after PHK infection with ***S. aureus***. ELISA plates were coated with the NET standard samples as well as with PMN supernatant over night at 4 °C. After washing 3 times with PBS containing 0.05% Tween-20, plates were blocked with PBS containing 2% BSA for 1 hour at room temperature. After washing 3 times, a 1:200 dilution of an anti-dsDNA antibody (Merck Millipore, clone 16-13) in PBS containing 0.5% BSA and 0.05% Tween-20 was added for 1 hour at room temperature. After another washing step, a 1:3000 dilution of an anti-mouse horseradish peroxidase conjugated antibody (Cell Signaling Technology) in PBS containing 0.5% BSA and 0.05% Tween-20 was added for 1 hour at room temperature. After washing, 100 µL of TMB substrate solution (Cell Signaling Technology) were added and the reaction was stopped with 50 µL H_2SO_4. The absorbance at 450 nm was measured using a Fluoroskan II (Labsystems). Samples were calculated in % of the NET standard.

NET isolation

NET isolation was adapted from (Najmeh et al., 2015). Briefly, $2x10^6$ PMNs per mL were treated with 500 nM PMA (Sigma) for 4 hours. Subsequently, remaining cells were gently removed, and NETs were gently washed off the tissue culture dish by cold PBS. After centrifugation (10 min, 450 g, 4 °C) supernatant was removed and centrifuged for 10 min at 18 000 g at 4 °C. Resulting pellets were resuspended in cold

water corresponding to an amount of $2x10^7$ PMNs. DNA concentrations were measured. Indicated concentrations of NETs were added to the lower compartment of the co-culture model instead of PMNs overnight.

Genomic DNA isolation

Isolated neutrophils from 10 mL blood were resuspended in 50 mM Tris –HCl (pH 8), 100 mM EDTA, 100 mM NaCl, 1% SDS. Upon overnight incubation at 55 °C with 10 mg/mL Proteinase K (Sigma) 700 µL phenol: chloroform (1:1) were added and mixed for 10 minutes. Subsequently, samples were centrifuged for 5 min at 13000 rpm. The aquatic phase along with the interface was transferred into a fresh tube. Phenol: chloroform was added again followed by another centrifugation step and overpassing of the aquatic phase. This step was repeated three times. In the following, the aquatic phase was mixed with 500 µL isopropanol until a stringy precipitate was formed. After centrifugation (10 seconds, 13 000 rpm) the supernatant was discarded and the pellet was dissolved in 70% EtOH and centrifuged for 2 minutes at 13 000 rpm. After drying, the pellet was resuspended in 200 µL keratinocyte base medium (CELLnTEC) and DNA content was measured after 1 hour.

Viability assay

Effects of NETs on primary human keratinocyte cell viability were tested using 4-methylumbelliferyl heptanoate (MUH). Therefore, keratinocytes were treated with indicated NET concentrations for 24 hours followed by incubation with 100 µg/mL MUH (Sigma-Aldrich) in PBS for 1 hour at 37°C. The absolute fluorescence intensity at λ_{ex} of 355 nm and λ_{em} of 460 nm was measured using a Fluoroskan II (Labsystems).

Confocal laser scanning microscopy

NET staining was adapted from (Brinkmann et al., 2010). $2x10^6$ PMNs per mL were seeded onto Poly-L-Lysine coated coverslips (BioCoat™, Corning) in 24-well plates

and subsequently infected with staphylococci (MOI=30). After 3.5 hours supernatant was carefully removed, and coverslips were fixed with 4% formaldehyde for 10 minutes at room temperature. Coverslips were then carefully removed from 24-well culture plate by lifting it up at the edge using a curved needle and fine forceps. Coverslips were washed three times upside down on a drop of PBS containing 0.05% BSA and 0.05% Tween-20, followed by blocking in 30% donkey serum (Sigma) for 30 min at 37 °C. Subsequently, coverslips were incubated on a drop of primary antibody dilution for 1 hour at 37 °C. Antibodies were goat-anti human MPO (RandD Systems, #AF3667, 1:50) and rabbit anti citH3 (abcam, #ab5103, 1:250). After washing three times coverslips were incubated on a drop of secondary antibody containing anti-rabbit-Alexa647 (Dianova, 1:500) and anti-goat-Cy3 (Dianova, 1:500) for 1 hour at 37 °C. After washing three times, nuclear staining was performed by monomeric cyanine nucleic acid stain (YO-PRO-1) (Invitrogen) for 5 minutes followed by three washing steps. Finally, coverslips were mounted onto glass slides using Mowiol 4-88 (Sigma). Confocal laserscanning microscopy was performed by using an LSM 800 (Zeiss).

Immunohistochemistry

For immunohistochemistry staining of mouse skin 3 µM tissue sections were de-paraffinized and antigen retrieval was performed in EDTA buffer at pH 9 (Thermo Fisher Scientific) in a pressure cooker for 5 min before a slow cooling down of the samples in the buffer. Afterwards, tissue sections were washed in PBS and blocked in 5% donkey serum in PBS containing 0.05% Triton X-100 for 90 minutes. Subsequently, tissue sections were incubated overnight at 4 °C in a humid chamber with a MPO-specific antibody (R and D Systems) diluted 1:50 in blocking buffer. The next day, tissue sections were washed and incubated with a 1:250 dilution of alkaline phosphatase-coupled secondary antibody (Novus Biologicals) for 90 minutes in a humid chamber at room temperature. After washing in PBS, staining was performed

by using the Lab Vision™ liquid fast red substrate system (Thermo Fisher Scientific) according to manufacturer's instructions. After washing in water hematoxylin-eosin staining (Agilent/Dako) was performed for 2 minutes. After another washing step in water tissue sections were mounted with Kaisers glycerine gelatine (Merck).

Quantification and statistical analysis

Significant differences between the means of the different treatments were evaluated using Graphpad Prism 7.0 and 8.0 (Graphpad Software, Inc.). Either unpaired, two-tailed Student's *t*-test or one-way ANOVA followed by Dunnett's, Sidak's or Tukey's multiple comparisons test was used for statistical analysis and indicated in the respective figure legends. Differences were considered statistically significant with a p value of less than 0.05. Data are visualized using Graphpad Prism 7.0 and 8.0 (Graphpad Software, Inc.), MS Excel (Microsoft Corporation) or FlowJo (Treestar).

Ethics statement

All mouse experiments were conducted in accordance with the German regulations of the Gesellschaft für Versuchstierkunde/Society for Laboratory Animal Science (GV-SOLAS) and the European Health Law of the Federation of Laboratory Animal Science Associations (FELASA). The protocol was approved by the Regierungspräsidium Tübingen (Permit Numbers: HT1/12; HT1/17). Keratinocyte isolation from human foreskin was approved by the ethics committee of the medical faculty of the University Tübingen (654/2014BO2) and performed according to the principles of the Declaration of Helsinki. Immune cell isolation from human blood was approved by the ethics committee of the medical faculty of the University of Tübingen (054/2017BO2). Foreskin and blood samples were anonymized and written consent was given to the physician in charge.

References

Bitschar, K., Sauer, B., Focken, J., Dehmer, H., Moos, S., Konnerth, M., Schilling, N.A., Grond, S., Kalbacher, H., Kurschus, F.C., *et al.* (2019). Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. Nat Commun ***10***, 2730.

Brinkmann, V., Laube, B., Abu Abed, U., Goosmann, C., and Zychlinsky, A. (2010). Neutrophil extracellular traps: how to generate and visualize them. J Vis Exp.

Burian, M., Bitschar, K., Dylus, B., Peschel, A., and Schittek, B. (2017). The Protective Effect of Microbiota on S. aureus Skin Colonization Depends on the Integrity of the Epithelial Barrier. J Invest Dermatol ***137***, 976-979.

Munzenmayer, L., Geiger, T., Daiber, E., Schulte, B., Autenrieth, S.E., Fraunholz, M., and Wolz, C. (2016). Influence of Sae-regulated and Agr-regulated factors on the escape of Staphylococcus aureus from human macrophages. Cell Microbiol ***18***, 1172-1183.

Najmeh, S., Cools-Lartigue, J., Giannias, B., Spicer, J., and Ferri, L.E. (2015). Simplified Human Neutrophil Extracellular Traps (NETs) Isolation and Handling. J Vis Exp.

Nguyen, M.T., Luqman, A., Bitschar, K., Hertlein, T., Dick, J., Ohlsen, K., Broker, B., Schittek, B., and Gotz, F. (2017). Staphylococcal (phospho)lipases promote biofilm formation and host cell invasion. Int J Med Microbiol.

Sil, P., Wicklum, H., Surell, C., and Rada, B. (2017). Macrophage-derived IL-1beta enhances monosodium urate crystal-triggered NET formation. Inflamm Res ***66***, 227-237.

Stoll, H., Dengjel, J., Nerz, C., and Gotz, F. (2005). Staphylococcus aureus deficient in lipidation of prelipoproteins is attenuated in growth and immune activation. Infect Immun ***73***, 2411-2423.

Whiteley, L., Meffert, T., Haug, M., Weidenmaier, C., Hopf, V., Bitschar, K., Schittek, B., Kohler, C., Steinmetz, I., West, T.E., *et al.* (2017). Entry, Intracellular Survival, and Multinucleated-Giant-Cell-Forming Activity of Burkholderia pseudomallei in Human Primary Phagocytic and Nonphagocytic Cells. Infect Immun ***85***.

Zipperer, A., Konnerth, M.C., Laux, C., Berscheid, A., Janek, D., Weidenmaier, C., Burian, M., Schilling, N.A., Slavetinsky, C., Marschal, M., *et al.* (2016). Human commensals producing a novel antibiotic impair pathogen colonization. Nature ***535***, 511-516.

Acknowledgements

This thesis is the culmination of my very personal PhD journey which was accompanied by hardship, success, fright and frustration. However, many people including family members, friends, colleagues, mentors and other people I have met on the way have contributed to accomplish this huge task. Without ALL of you this work would not have been possible!

First of all, I would like to express my deepest gratitude to my supervisor Prof. Dr. Birgit Schittek for accepting me as her PhD student and giving me the opportunity to work on this very timely project. Thank you very much for your trust that allowed me to independently work on this project and to follow my own ideas. At the same time, from the start you were always available offering me your help and guidance when I needed it.

Next, I want to thank Prof. Dr. Andreas Peschel for helpful cooperation, advice, ideas and for being my second PhD supervisor.

My earnest thanks to ALL my co-authors, without ALL of you these publications would not have been possible. I especially want to mention here two of my students: Jule and Laura. Thank you for working as hard as you did. I know it can be hard working according to my fastidious ideas and wishes. But nevertheless, you provided such high-quality data for me. Additionally, I want to thank Bibo for her general assistance and for performing the immunohistochemical staining for me. Moreover, I want to thank Sonja Moos, for sharing her immunology knowledge and techniques with me. Special thanks to Bernhard Krismer, who always provided new bacterial strains as well as his vast *S. aureus* knowledge. Furthermore, I want to thank Birgit Fehrenbacher for sharing her expertise in microscopy.

I would like to express my gratitude to all the people who are part of the TR156. Thanks to all for the fruitful exchanges, cooperations, unforgettable social events and valuable assistance at our retreats and youngster events. I am very happy to have met all of you and to have been part of this scientific community!

Moreover, I want to thank all my current and former colleagues in the lab: Elena, Heike, Corinna, Tobias, Francisco, Bibo, Jule, Laura, Janina, Hanna, Ilknur, Ines,

Franziska, Emanuel, Marc and Theresa. Thank you all for your valuable advice and of course for fun coffee and lunch breaks during the last years.

Finally, I want to acknowledge the people who mean the most to me: I wish to thank my friends and family for their continuous support and encouragement throughout this journey. Thank you for constantly reminding me that there is life outside the lab. All of you provided the distraction I needed to stay on track. Vielen Dank Mama und Papa, dass ihr immer an mich glaubt und mich bei allem unterstützt. I want to express the most special thanks to my own little family: Thank you, Nils, for your continuous support and understanding. You were always around after a hard day at work and long hours stuck in traffic. And to my daughter, who joined us during the process of writing this thesis: Thank you, Anni, for unlimited joy and happiness and for giving me the time to finalize this thesis. Words cannot express how grateful I am to both of you.