Review article

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

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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 immune cells orchestrate an effective defense against *S. aureus* in healthy skin and the mechanisms leading to an impaired keratinocyte function in AD patients.

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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.

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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 (CLR) [1,2]. TLRs are the best characterized human PRRs [3,4]. They recognize conserved microbial structures such as lipopolysaccharide (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]. TLR2 and NOD2 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-κB1ζ (also called MAIL or IκBζ), which either enhances or suppresses transcription of NF-κB target genes depending on the cell type [16]. NF-κB1ζ is constitutively expressed in keratinocytes, but expression can be enhanced by TLR ligands or IL-1. Interestingly, knockout of NF-κB1ζ in mouse skin led to elevated levels of proinflammatory cytokines and chemokines and severe dermatitis resembling AD pathology [17]. These data suggest that NF-κB1ζ 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]. Ubiquitylation 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 HIIFα 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 inflammatory some, 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]. These 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]. Intriguing, recent evidence shows that antimicrobial peptide-producing coagulase-negative staphylococci can be missing in AD and reconstitution 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 stratum 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.
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 innate 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.

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