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Host and microbe determinants that may influence the success of *S. aureus* colonization

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Staphylococcus aureus may cause serious skin and soft tissue infections, deep abscesses, endocarditis, osteomyelitis, pneumonia, and sepsis. *S. aureus* persistently colonizes 25–30% of the adult human population, and *S. aureus* carriers have an increased risk for infections caused by the bacterium. The major site of colonization is the nose, i.e., the vestibulum nasi, which is covered with ordinary skin and hair follicles. Several host and microbe determinants are assumed to be associated with colonization. These include the presence and expression level of bacterial adhesins, which can adhere to various proteins in the extracellular matrix or on the cellular surface of human skin. The host expresses several antimicrobial peptides and lipids. The level of β-defensin 3, free sphingosine, and *cis*-6-hexadecenoic acid are found to be associated with nasal carriage of *S. aureus*. Other host factors are certain polymorphisms in Toll-like receptor 2, mannose-binding lectin, C-reactive protein, glucocorticoid-, and vitamin D receptor. Additional putative determinants for carriage include genetic variation and expression of microbial surface components recognizing adhesive matrix molecules and their interaction partners, as well as variation among humans in the ability of recognizing and responding appropriately to the bacteria. Moreover, the available microflora may influence the success of *S. aureus* colonization. In conclusion, colonization is a complex interplay between the bacteria and its host. Several bacterial and host factors are involved, and an increased molecular understanding of these are needed.

Keywords: *Staphylococcus aureus*, colonization, MSCRAMM, PAMP, PRR, immune evasion, microflora

INTRODUCTION

Human skin is easily accessible for microbial colonization and provides a wide variety of environmental conditions for growth. One of our most potent pathogens, *Staphylococcus aureus*, is a ubiquitous commensal that persistently colonizes 25–30% of the human population (Wertheim et al., 2004; van Belkum, 2011). *S. aureus* is the major cause of skin and soft tissue infections, and the bacteria can also infect any tissue of the body, causing other serious or life-threatening diseases, such as deep abscesses, endocarditis, osteomyelitis, and pneumonia. *S. aureus* is one of the most prevalent pathogens in bloodstream infections and effective treatment is a major clinical challenge. Emergence and spread of antimicrobial resistance combined with increasing numbers of immune-compromised patients make infections increasingly difficult to treat (Tong et al., 2012). Infection rates are higher in carriers of *S. aureus* than in non-carriers and invasive disease is often caused by the strain carried by the patient (von Eiff et al., 2001a; Wertheim et al., 2004). Eradication of nasal colonization with antibiotic treatment decreases the risk of post-operative infections (van Rijen et al., 2008), which may support an approach in which systemic *S. aureus* infections are prevented by eliminating or reducing nasal carriage.

Historically, individuals have been classified as non-carriers, intermittent carriers, or persistent carriers of *S. aureus*. However, non-carriers and intermittent carriers share similar nasal elimination kinetics and anti-staphylococcal antibody profiles. Therefore, a reclassification of *S. aureus* nasal carriage is suggested and includes only two types of nasal carriers: persistent or others (van Belkum et al., 2009). The persistent carriage rate varies depending on age, gender, serum glucose level, smoking, oral contraceptive use, dialysis, and/or drug addiction (Kluytmans et al., 1997; Choi et al., 2006; Olsen et al., 2009; van Belkum, 2011). Various diseases are also associated with increased carriage rate, such as Wegener's granulomatosis patients with 63–72%, atopic dermatitis (AD) patients with almost 100% and rheumatoid arthritis patients with 50% carriage rate (Breuer et al., 2002; Laudien et al., 2010). Still, the success of *S. aureus* to colonize human nares remains an enigma. Since not all humans are persistent carriers and some bacterial clones are more common than others within a population, both human and microbial determinants seem to be involved. In this paper, we intend to review and discuss various parameters that may contribute to nasal carriage.

STAPHYLOCOCCUS AUREUS COLONIZATION OF THE HUMAN HOST

LOCALIZATION OF *S. AUREUS*

Staphylococcus aureus has been found in human nose, throat, perineum, and intestine (Wertheim et al., 2005; Mertz et al., 2007; Acton et al., 2009). However, as decolonization of the nose usually has a decolonizing effect on perineum, pharynx, and axillae as well, the nose is assumed to be the major site of *S. aureus* colonization (Kluytmans and Wertheim, 2005) although sole intestinal

carriage also can occur (Acton et al., 2009). Sampling of the various areas inside the noses of healthy individuals revealed that *S. aureus* is mainly localized in the vestibulum nasi (Cole et al., 2001), and the bacterial load of two persistent carriers varied between 10^4 and 10^5 bacteria/nasal swab over time (Burian et al., 2010b).

Intracellular *S. aureus* have been found in biopsies from the anterior part of the middle turbinate or tonsils from patients with recurrent rhinosinusitis or tonsillitis, respectively (Clement et al., 2005; Zautner et al., 2010). The intracellular residency in epithelial cells was a significant risk factor for recurrent episodes of rhinosinusitis (Plouin-Gaudon et al., 2006). The intracellular localization of *S. aureus* is thought to be part of the pathophysiological mechanisms behind prolonged course, chronic, or frequent relapse of rhinosinusitis, osteomyelitis, mastitis, or endocarditis (Plouin-Gaudon et al., 2006; Garzoni and Kelley, 2009; Sinha and Fraunholz, 2010). *S. aureus* can be internalized by various cell types, such as endothelial cells, epithelial cells, fibroblasts, osteoblasts, and keratinocytes in addition to the professional phagocytes (Garzoni and Kelley, 2009). The invasiveness and survival of bacteria within the mammalian cells varies, and may depend on bacterial strain, ability to form small colony variant (SCV), multiplicity of infection (MOI) as well as the mammalian cell type. Prolonged survival of bacterial cells can result in release of viable bacterial cells when the host cell dies (von Eiff et al., 2001b; Krut et al., 2003; Garzoni and Kelley, 2009; Sinha and Fraunholz, 2010). Whether *S. aureus* can be found intracellular within keratinocytes during nasal colonization remains unknown, but *S. aureus* is rapidly invading the keratinocytes in skin biopsies taken from human hosts (Kisich et al., 2007).

FEATURES OF THE COLONIZED TISSUE

A histological study of human cadavers revealed bacteria in stratified squamous epithelium and hair follicle shafts in the nose (Ten Broeke-Smits et al., 2010). The outermost area in the nose is covered by ordinary skin, which is divided into two main structures: a layer of highly regenerating epidermis and dermis, which is drained by lymphatic and vascular conduits (Nestle et al., 2009). The dominating cell type in epidermis is keratinocytes, but also Langerhans cells, melanocytes, Merkel cells, and T cells are present (Nestle et al., 2009; Hari et al., 2010). The keratinocytes in the basal layer (stratum basale) express the basal keratins K5, K14, and K15. Through continuous proliferation, the basal layer provides cells that differentiate, move, and form the stratum spinosum. The postmitotic cells in stratum spinosum express suprabasal keratins K1 and K10, which reinforce mechanical strength of the layer. These cells continue to differentiate and form stratum granulosum. The keratinocytes in stratum granulosum contain lamellar bodies, which are secretory organelles that are unique to epidermis. In the terminal differentiation of keratinocytes, filaggrin aggregates the keratin filaments into tight bundles, promoting the collapse of the cells. The resulting multilayer of flattened, anucleated corneocytes is called stratum corneum or the cornified layer. Corneocytes contain a cornified envelope consisting of structural proteins such as involucrin, loricrin, trichohyalin, transglutaminases, and filaggrin in addition to keratin K1 and K10 providing mechanical strength. A complex series of lipids are covalently attached to the proteins of the cornified envelope. Moreover, the laminar bodies secrete hydrolytic enzymes as well as phospholipids, ceramides, glycosyl ceramides, and sterols, which can be further metabolized in the extracellular space (Proksch et al., 2008; Nestle et al., 2009). Additional lipids are provided by the sebaceous glands (Toth et al., 2011).

Almost all patients with AD are colonized with *S. aureus* (Breuer et al., 2002). Loss-of-function variants of the structural epidermal protein filaggrin are present in 9% of European population and are found to be predisposing factor for AD (Palmer et al., 2006). Individuals with loss-of-function filaggrin have significantly reduced natural moisturizing factor in stratum corneum and higher transepidermal water loss (Kezic et al., 2008). The components of natural moisturizing factor include urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA), which in addition to hydration may also be involved in pH regulation of the skin. UCA and PCA are breakdown products from filaggrin. Physiological concentrations of these compounds reduce the growth rate of *S. aureus* as well as the expression of bacterial proteins involved in colonization and immune evasion. This suggests that colonization of individuals with loss-of-function mutations of filaggrin, which lacks the breakdown products in skin, may have a reduced ability to inhibit *S. aureus* growth (Miajlovic et al., 2010).

BACTERIAL AND HOST PROTEINS INVOLVED IN ADHESION

The adherence between *S. aureus* and the nasal epithelium is a multifactorial process that involves various interaction partners in both organisms. The primary bacterial adherence is thought to be mediated by wall teichoic acid (WTA), whereas sortase-anchored microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) have critical roles at later stages of colonization of the human nose (Weidenmaier et al., 2008; Burian et al., 2010a). Detailed MSCRAMM-host interaction *in vitro* studies have revealed that *S. aureus* clumping factor B (ClfB) adheres to cytokeratin K10 (O'Brien et al., 2002; Wertheim et al., 2008; Clarke et al., 2009) and K8 (Haim et al., 2010). ClfB is expressed during nasal colonization and contributes to nasal colonization in humans (Wertheim et al., 2008; Burian et al., 2010b). However, ClfB-deficient cells of *S. aureus* strains 8325-4 and Newman could still interact with the nasal cells (O'Brien et al., 2002), which clearly demonstrate the utilization of several independent MSCRAMMs for colonization.

Another *S. aureus* adhesin that has shown interactions with harvested human desquamated epithelial cells is Iron-regulated surface determinant A (IsdA) (Clarke et al., 2006), which is also expressed during nasal colonization in humans (Burian et al., 2010b). The corneocyte envelope proteins loricrin and involucrin are identified as IsdA interaction partners (Clarke et al., 2009). The bacterial proteins SdrC, SdrD, and SasG are also shown to promote adhesion of bacteria to desquamated nasal epithelial cells harvested from human donors (Roche et al., 2003; Corrigan et al., 2009). Several other *S. aureus* MSCRAMMs or adhesins are identified, which interact with host molecules such as fibrinogen, elastin, collagen, or von Willebrand factor (Heilmann, 2011). However, the involvement of these factors in nasal colonization remains elusive.

The panel of MSCRAMMs varies among *S. aureus* isolates (McCarthy and Lindsay, 2010). Interestingly, comparison of *S. aureus* 8325-4 and *S. aureus* 8325-4 (pKS80::*sasG*) showed that expression of SasG reduced *S. aureus* adherence to fibrinogen and fibronectin. Thus, certain MSCRAMMs might mask the adhesive functions of other adhesins, and possibly even change the tropism for the host cell (Roche et al., 2003). The individual combinations of MSCRAMMs may therefore influence the microbial success of adhesion. Also, allelic variations between bacterial adhesins of the same type may also either improve or reduce their binding to human cells. Moreover, there may be variations in expression level of MSCRAMMs. Different MSCRAMM expression profiles were, for example, observed for so-called carrier and non-carrier strains of *S. aureus*, where the carrier strain expressed a markedly larger number of adhesive proteins such as SasD and SdrH (Muthukrishnan et al., 2011).

Cells from different donors provide variable levels of adhesion to *S. aureus* (Aly et al., 1977; Weidenmaier et al., 2008). This may suggest that there are differences in the expression levels or different polymorphic variants of human interaction partner, which may influence the level of bacterial adhesion. Fibronectin and fibrinogen contribute to *S. aureus* binding. Fibronectin has been found in stratum corneum of AD skin, but not in healthy skin, which may partly explain the abundant colonization of atopic skin (Cho et al., 2001). Also, AD patients have an impaired proliferation and differentiation in both non-lesional and lesional skin, as seen by immunohistochemistry of Ki67 (a marker of cell proliferation; Scholzen and Gerdes, 2000), involucrin, loricrin, filaggrin, and the keratins K5, K6, K10, K16, and K17 (Jensen et al., 2004). Loricrin, involucrin, and K10 are interaction partners for ClfB and/or IsdA, and aberrant distribution of the interaction partners of these may be beneficial for colonization. Finally, there has also been found amino acid variation in elastin, fibrinogen/fibrin, fibronectin, prothrombin, vitronectin, and von Willebrand factor (McCarthy and Lindsay, 2010). Such an amino acid variation may also influence the risk of colonization.

All these studies suggest that the success of adhesion to host may depend on the correct combination and allelic variant of MSCRAMM together with the appropriate expression and variant of human host ligand.

EXPRESSION OF COLONIZATION DETERMINANTS

Burian et al. (2010b) addressed the expression of several *S. aureus* transcripts during nasal colonization of humans. The bacteria were found to express the adhesive molecules (*clf*B, *isd*A, *fnb*A, *atl*A, *eap*, WTA), genes involved in cell surface dynamics/remodeling (*sce*D, *oat*A, *atl*A) and immune-modulatory factors (*sak*, *chp*, *spa*, *eap*) under these conditions. In contrast, the major toxins (*hla*, *psm*) were not transcribed during colonization of the human nose.

The environment in the nose may also require reactive oxygen species and desiccation tolerance. Alkyl hydroperoxide reductase (*AhpC*) and catalase (*katA*), which engender increased H_2O_2 tolerance, were found to be associated with nasal carriage in a cotton rat model (Cosgrove et al., 2007), but their possible impact on colonization of the human nose is not known.

INFLUENCE OF BIOFILM

Biofilm and planktonic cultures of *S. aureus* have different impacts on gene expression in keratinocytes (Secor et al., 2011). In nasal secretion, a so-called carrier strain of *S. aureus*, but not a non-carrier strain, was surrounded by an additional electron-dense layer covering the peptidoglycan, which might represent the initial stages of biofilm formation (Cole et al., 2001; Quinn et al., 2009). This may provide resistance against phagocytosis and/or oxidative burst. Moreover, the carrier strain biofilm exoproteome contained a greater number of immunoevasive proteins than its planktonic counterpart (Muthukrishnan et al., 2011). However, a dispersed rather than biofilm-related mode of growth is thought to occur during *S. aureus* nasal colonization (Krismer and Peschel, 2011).

THE INFLUENCE OF MICROFLORA ON *S. AUREUS* NASAL CARRIAGE

The nares are colonized by a temporally stable microbiota distinct from other regions of the integument (Frank et al., 2010). The nasal microbiota of healthy adults has been shown to consist primarily of members of the phylum Actinobacteria (e.g., *Propionibacterium* spp. and *Corynebacterium* spp.), but also Firmicutes (e.g., *Staphylococcus* spp.) and Proteobacteria (e.g., *Enterobacter* spp.) (Frank et al., 2010; Wos-Oxley et al., 2010). Healthy adults harbor significantly more species-rich and diverse nares microbiotas than hospitalized individuals (Wos-Oxley et al., 2010).

Staphylococcus epidermidis is the most commonly isolated bacterial species from healthy human skin and may protect humans from pathogenic bacteria. *S. epidermidis* produce phenol-soluble modulins, which inhibit the growth of *Streptococcus pyogenes*. These modulins also induce lipid vesicle leakage and exert antimicrobial action against *S. aureus* (Cogen et al., 2010a,b). In addition, Lai et al. (2009) showed that the microflora can modulate specific cutaneous inflammatory responses and that a product of staphylococci, lipoteichoic acid (LTA), inhibits skin inflammation. Moreover, *S. epidermidis* LTA modulates the inflammation through a Toll-like receptor (TLR)-cross-talk phenomenon between TLR2 and TLR3. Finally, the presence of *S. epidermidis* has been shown to induce expression of human β-defensin 2 (hBD2) and human β-defensin 3 (hBD3) in keratinocytes, thereby inhibiting the growth of pathogenic organisms, e.g., *S. aureus* and group A streptococci (GAS) (Lai et al., 2010).

Staphylococcus aureus carriage has been shown to be negatively associated with a variety of other nares-associated microbial species, most significantly *S. epidermidis* and *Propionibacterium acne* (Frank et al., 2010). Bogaert et al. (2004) noted a negative correlation for co-colonization of *S. aureus* and vaccine-type pneumococci in the nasopharynx of children, but not for *S. aureus* and non-vaccine serotype pneumococci, suggesting that there might be a natural competition between colonization with vaccine-type pneumococci and *S. aureus*.

Uehara et al. (2000) reported a low incidence of *S. aureus* carriage in individuals who also were positive for *Corynebacterium* sp. by nose swabs suggesting competition for survival between *S. aureus* and corynebacteria. Lina et al. (2003) later showed that the *S. aureus* nasal colonization rate correlated negatively with the rate of colonization by *Corynebacterium* spp. and *S. epidermidis*, suggesting that both *Corynebacterium* spp. and

S. epidermidis antagonize *S. aureus* colonization. Colonization by methicillin-resistant *S. aureus* (MRSA) agr-1Sa strains was specifically associated with a low rate of colonization by *Corynebacterium* spp. and agr-3Se *S. epidermidis* (Lina et al., 2003), thus suggesting a potential competitive interaction among different species with special emphasis on the influence of staphylococcal *agr* alleles.

Staphylococcus epidermidis may also "protect" the host from *S. aureus* colonization via quorum sensing cross-inhibition. Production of specific peptide pheromones affect *agr* signaling in competing bacteria, and thus lead to colonization inhibition of, e.g., *S. aureus* (Otto et al., 2001). A serine protease (Esp, 27 kDa) secreted by a subset of *S. epidermidis* can inhibit *S. aureus* biofilm formation and nasal colonization *in vivo*. *S. epidermidis* cells were introduced into the nasal cavities of volunteers who were *S. aureus* carriers. The wild-type *S. epidermidis* eliminated *S. aureus* colonization, but its isogenic *esp* mutant did not (Iwase et al., 2010).

The displacement of *S. aureus* by *S. pneumoniae* in the nasopharynx may be explained by H_2O_2-mediated bacterial interference. Hydrogen peroxide produced by *S. pneumoniae* kills lysogenic but not non-lysogenic staphylococci by inducing the SOS response. The SOS response induces resident prophages and thereby lysis and H_2O_2 lethality (Selva et al., 2009). It has also been shown that production of H_2O_2 by viridans group streptococci may inhibit MRSA colonization of oral cavities in newborns (Uehara et al., 2001). But in mixed-inoculum colonization experiments and experiments where *S. aureus* invaded the nasopharynx of rats with established *S. pneumoniae* populations, the density of *S. aureus* did not differ whether the *S. pneumoniae* strain was H_2O_2 secreting or non-H_2O_2 secreting (Margolis et al., 2010).

HOST MOLECULES THAT MIGHT INFLUENCE *S. AUREUS* CARRIAGE

Staphylococcus aureus colonizes the human nares in an area covered with ordinary skin supplemented with nasal secretions. Thus, host molecules that might influence *S. aureus* carriage should be constitutively expressed and/or induced in the skin or nasal secretions. In the following, the focus will be on antimicrobial molecules, various cytokines, and the signaling pathways involved in the induction of them. In addition, we will provide examples of known polymorphisms in the signal molecules and complement factors/regulators that may influence the host defense against colonization or infection against *S. aureus*. There will also be a brief discussion about the adaptive immune system and its contribution to host protection against *S. aureus* colonization.

ANTIMICROBIAL MOLECULES AND COLONIZATION

The epidermis contains several antimicrobial lipids, peptides, or proteins provided by keratinocytes, sebocytes, mast cells, and eccrine sweat glands and also by circulating neutrophils or natural killer cells that are recruited to the skin (Schauber and Gallo, 2009). The various antimicrobial molecules may act in synergy to prevent colonization (Proksch et al., 2008), and their individual or combined expression pattern may influence the colonization status of *S. aureus*.

Lipids

Lipids in epidermis that have antimicrobial activity against *S. aureus* are the fatty acids lauric acid, sapienic acid, oleic acid, palmitoleic acid, and *cis*-6-hexadecenoic acid (Wille and Kydonieus, 2003; Takigawa et al., 2005; Nakatsuji et al., 2009; Chen et al., 2011; Toth et al., 2011). Similarly, sphingoid bases are synthesized from ceramides, and have broad antibacterial and antifungal activities (Proksch et al., 2008). A reduction in free sphingosine and *cis*-6-hexadecenoic acid has been found to be associated with carriage of *S. aureus* among AD patients (Arikawa et al., 2002; Takigawa et al., 2005). However, whether this pattern is found among healthy carriers is unknown.

Antimicrobial peptides/proteins

Another important player in the first-line defense in skin are antimicrobial peptides (AMPs) or proteins (Yamasaki and Gallo, 2008; Wiesner and Vilcinskas, 2010). Production of AMPs may be constitutive or induced by inflammation or injury. The various tissues produce specific profiles of different AMPs, which may vary significantly depending on the physiological condition. AMPs have the ability to kill the pathogens directly, and also to initiate a host defense response by signaling via receptors resulting in production of chemokines and recruitment of immune cells (Yamasaki and Gallo, 2008; Wiesner and Vilcinskas, 2010). In the following, we intend to provide a few examples of AMPs and proteins, and describe what is known regarding them and colonization with *S. aureus*.

Human α-defensins, also called human neutrophil peptides, include the peptides HNP 1–4 and HD 5–6. The former are expressed in neutrophils, while the latter two peptides are mainly expressed in Paneth cells in the small intestine. The human β-defensins (hBD1–4) are expressed in mucosa and epithelial cells (Yamasaki and Gallo, 2008; Wiesner and Vilcinskas, 2010). The β-defensins have been compared in their antimicrobial activity against *S. aureus*, and the most potent is β-defensin 3 followed by β-defensin 2 and β-defensin 1 (Midorikawa et al., 2003; Chen et al., 2005; Kisich et al., 2007). Keratinocytes have constitutive capacity to kill *S. aureus*, a phenomenon that is dependent on β-defensin 3 (Kisich et al., 2007). Comparisons of skin biopsies revealed that the levels of β-defensin 3 are similar between normal individuals and those with AD. However, the AD patients have a reduced ability to mobilize β-defensin 3 onto the bacteria due to presence of Th2 cytokines (Kisich et al., 2008). Also, a higher induction of β-defensin 3, but not β-defensin 2, is associated with a better clinical course and outcome of *S. aureus* skin infections (Zanger et al., 2010). Moreover, the level of both constitutive and induced β-defensin 3 is lower in persistent *S. aureus* nasal carriers compared to non-carriers, suggesting that β-defensin 3 is a determinant for carriage (Zanger et al., 2011). All these studies show the importance of presence of β-defensin 3 in clearing of *S. aureus* in colonization and infection.

The expression level of defensins may be regulated at several stages. The genes encoding β-defensins are clustered in chromosome 8p23.1, which is a frequent site of genetic rearrangement, and copy numbers of defensin genes may therefore vary among individuals (Linzmeier et al., 1999). However, no associations between nasal carriage of *S. aureus* and copy number and/or sequence

variations among HNP 1–3 or β-defensin 3 have been found (van Belkum et al., 2007; Fode et al., 2011). Both α- and β-defensins are secreted as proproteins, and post-translational processing is needed in order to create mature defensins (Yamasaki and Gallo, 2008). The defensins' processing enzymes in human skin are unknown, and whether the activity of these influence carriage status is also unknown.

Cathelicidin is another AMP that is important in skin. The protein is stored as a proprotein named hCAP18 in laminar bodies in keratinocytes and secreted into the spinous layer of epidermis, where local proteases process the protein into active peptide(s). Stratum corneum tryptic enzyme (SCTE) modifies the propeptide into LL-37 at the skin surface. Then, a combination of SCTE and stratum corneum chymotryptic protease (SCCE) may further process LL-37 into smaller peptides known as RK-31 and KS-30 (Yamasaki and Gallo, 2008). RK-31 and KS-30 have increased antimicrobial activity against *S. aureus* compared to LL-37 (Murakami et al., 2004). Whether the level and/or function of SCTE/SCCE and/or level of the processed peptides are associated with nasal carriage is not known.

Vitamin D appears to promote with both innate and adaptive immunity. The active variant 1,25-$(OH)_2$-D_3 has been found to enhance the antimicrobial function against *S. aureus in vitro* (Schauber et al., 2007, 2008). The promoter of the antimicrobial proteins cathelicidin and β-defensin 2 have vitamin D responsive elements (VDREs) (Gombart et al., 2005), and 1,25-$(OH)_2$-D_3 can induce cathelicidin and/or β-defensin 2 in isolated human keratinocytes, monocytes, neutrophils, and myeloid cells as well as in human skin biopsies (Wang et al., 2004; Gombart et al., 2005; Weber et al., 2005). Also, supplementation of oral vitamin D induced cathelicidin production in AD lesional skin (Hata et al., 2008). In addition to inducing AMP expression, 1,25-$(OH)_2$-D_3 has been found to induce CD14 and TLR2 expression in keratinocytes (Schauber et al., 2007), which may result in increased ability to detect pathogens. Epidemiological studies suggest that polymorphism in vitamin D responsive genes might be associated with *S. aureus* carriage among type I diabetes patients (Panierakis et al., 2009), but not among a healthy, elderly populations (Claassen et al., 2005). There is also reported an association between vitamin D deficiency and MRSA nasal carriage (Matheson et al., 2010). Recently, we found that nasal carriage of *S. aureus* is associated with serum 25-hydroxyvitamin D level, gender, and smoking status (Olsen et al., 2011). All these studies suggest that the vitamin D protects against carriage of *S. aureus*, which may be due to, among other factors, increased expression of cathelicidin in the skin.

RNase 7 is an additional AMP found in stratum corneum. Lower RNase 7 expression in healthy skin is associated with increased risk for new onset of *S. aureus* skin infection (Zanger et al., 2009). However, no association was found between RNase 7 level and carriage of *S. aureus* (Zanger et al., 2011).

Finally, there are several other proteins that may have antimicrobial activity against *S. aureus*. Examples include dermcidin and histone H4, which are expressed in eccrine sweat glands and by sebocytes, respectively, and secreted to the skin (Rieg et al., 2004; Lee et al., 2009). Moreover, secretory leukocyte protease inhibitor (SLPI) and elafin/elastase-specific inhibitor (ESI)/skin-derived antileukoprotease (SKALP) kill *S. aureus* (Hiemstra et al., 1996; Simpson et al., 1999), and the proteins can be induced in keratinocytes/epidermis (Bando et al., 2007). Another class of putative relevant antimicrobial proteins is the peptidoglycan recognition proteins (PGRPs; Dziarski and Gupta, 2010). Furthermore, several cytokines and chemokines are found to have antimicrobial activity against *S. aureus* to various degrees: CXCL1/Groα, CXCL2/Groβ, CXCL3/Groγ, CXCL9/MIG, CXCL10/IP-10, CXCL11/I-TAC, CXCL12/SDF-1, CXCL13/BCA-1, CXCL-14/BRAK, XCL1/lymphotactin, CCL1/I-309, CCL11/eotaxin, CCL17/TARC, CCL18/PARC, CCL20/MIP3-α, CCL21/SLC, CCL22/MDC, CCL25/TECK, IL-1α, and IL-1β (Yang et al., 2003; Quinn et al., 2009). However whether the combination or expression level of any of the mentioned proteins influence carriage is unknown.

Nasal secretion and its effectors

Several proteins have been identified in nasal secretions, among them α-defensin 1–2, β-defensin 1–2, lysozyme, lactoferrin, and hemoglobin (Cole et al., 1999; Pynnonen et al., 2011). Carriers of *S. aureus* have elevated levels of α-defensins 1–3, β-defensin 2, lactoferrin, and sIgA in their nasal secretions (Cole et al., 2001; Kartashova et al., 2009). As α-defensins 1–3 are expressed in neutrophils, the elevated level may indicate the presence and/or activation of neutrophils in nasal secretion or epidermis among carriers. However, this remains to be investigated. The presence of hemoglobin in nasal secretions was recently found to be another host determinant for *S. aureus* colonization. The mechanism may involve hemoglobin-mediated inhibition of the expression of the *agr* quorum sensing system (Pynnonen et al., 2011).

THE HOST IMMUNITY AND *S. AUREUS* CARRIAGE

Bacteria contain or may secrete substances that are recognized by the host as pathogen-associated molecular patterns (PAMPs). The bacterial cell wall of *S. aureus* is composed of multiple peptidoglycan layers in combination with WTAs, LTA, and various MSCRAMMs. Moreover, the bacteria secrete *N*-formylated methionine and/or other substances that can be recognized as PAMPs (Fournier and Philpott, 2005). The cells involved in the innate immune system express various pattern-recognition receptors (PRRs), which specifically recognize PAMPs (**Figure 1**). Among the PRRs known to be involved in recognizing *S. aureus* is the TLR2 in various combinations with TLR1 or TLR6 and/or CD36 and CD14 (Fournier and Philpott, 2005; Nilsen et al., 2008) as well as the intracellular nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) NOD2 and NLRP3 (Fournier and Philpott, 2005; Craven et al., 2009). Activation of PRRs activates intracellular signaling resulting in altered gene expression (**Figure 1**). In addition, mannose-binding lectin (MBL) or ficollins may also bind to pathogens, thereby activating the complement system followed by opsonization and increased phagocytosis of the bacteria (Casanova and Abel, 2004).

Signaling through TLR

Toll-like receptor is a transmembrane glycoprotein, which consists of an extracellular domain, a transmembrane domain, and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain. Ligand binding

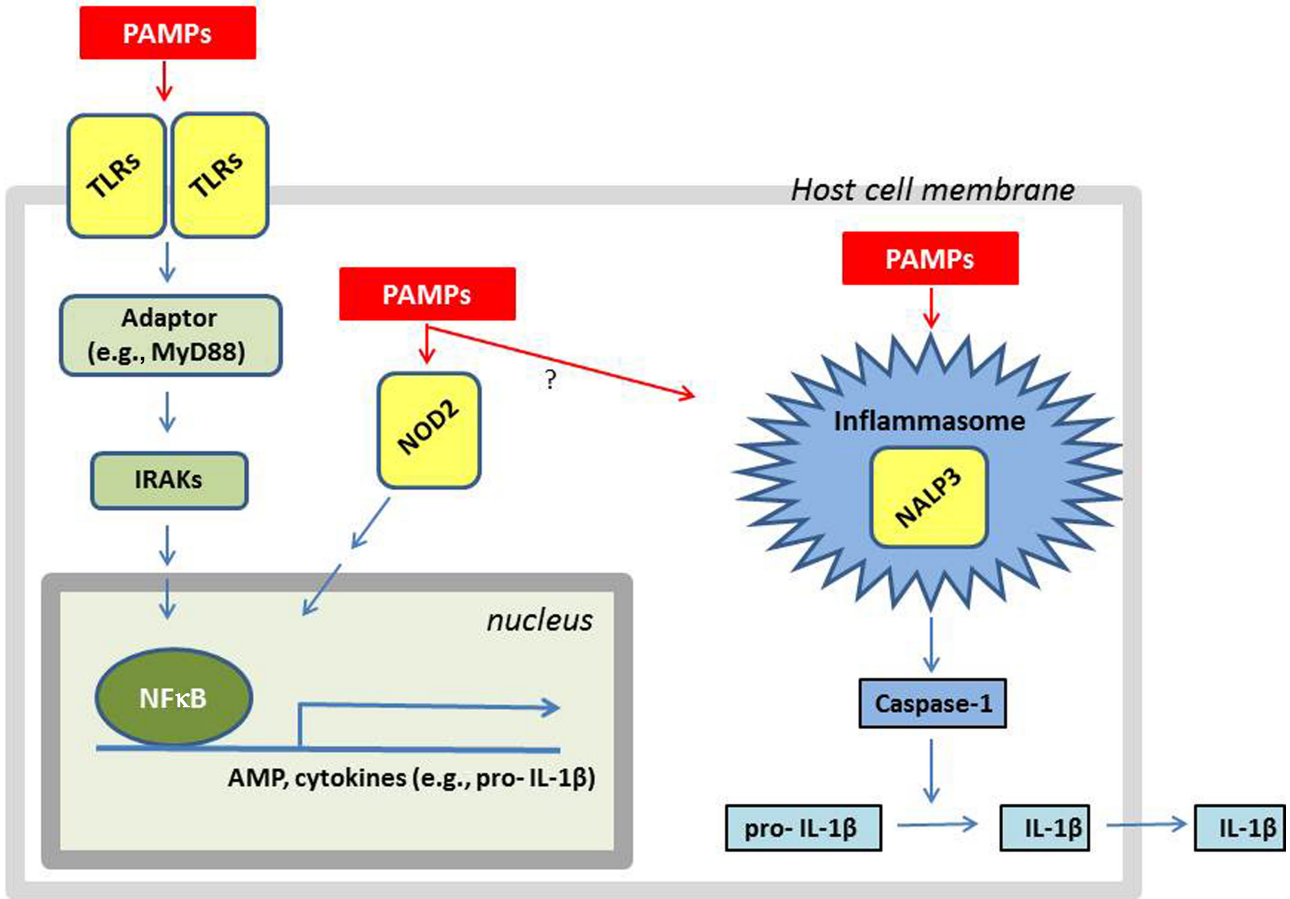

FIGURE 1 | A simplified overview of how a cell responds to the presence of *S.* aureus components. LTA and lipoproteins are recognized as PAMPs by cellular membrane bound TLRs such as TLR2:TLR1 or TLR2:TLR6. The activation of TLRs results in recruitment of adaptors (e.g., MyD88) which via IRAKs activate signal cascades resulting in nuclear translocation of the transcription factor NFκB. The nuclear localized NFκB stimulates the transcription of AMPs and cytokines (e.g., IL-1β). Breakdown products from peptidoglycan (e.g., muramyl dipeptide) and other bacterial proteins (e.g., α-hemolysin) are recognized as PAMPs by the intracellular receptors NOD2 and NALP3, respectively. The activation of NALP3 results in the assembly of the inflammasome, resulting in caspase-1 activation, followed by IL-1β processing and secretion. The activation of NOD2 can result in activation of NFκB resulting in AMP production, and perhaps also inflammasome assembly. Since little is known about intracellular signaling induced by MSCRAMMs, they were not included in the figure even though some of their interaction partners are known.

to TLRs results in recruitment of other TIR-containing proteins such as the adaptor protein myeloid differentiation primary-response protein 88 (MyD88), TIR-domain-containing adaptor protein (TIRAP), TIPAP-inducing interferon (IFN)-β (TRIF) or TRIF-related adaptor molecule (TRAM). This initiates a signaling cascade involving IL-1R-associated kinases (IRAKs) and other kinases, which activates mitogen-activated kinases (MAPKs) and transcription factors such as nuclear factor κB (NFκB) and IFN-regulatory factors (IRFs). The final result of the activation of the cascade is increased expression and secretion of various AMPs, cytokines, and chemokines, thereby recruiting immune cells to the site of infection and triggering of the adaptive immune response (Akira and Takeda, 2004; Kumar et al., 2009).

Subcutaneous inoculation of *S. aureus* into TLR2-, MyD88-, and IL-1R-deficient mice revealed that IL-1R/MyD88 signaling in resident skin cells were of particular importance in neutrophil recruitment to the site of infection (Miller et al., 2006). IL-1R ligands include IL-1α and IL-1β. Studies in mice showed that bone marrow cell-derived IL-1β, but not IL-1α, was found to be important for IL-1R-induced neutrophil recruitment against cutaneous *S. aureus* challenge *in vivo* (Miller et al., 2007). In humans, treatment of rheumatoid arthritis with IL-1 receptor antagonists results in increased susceptibility to *S. aureus* infections (Miller and Cho, 2011), but whether IL-1 receptor signaling is of importance in protecting against colonization is not known.

Autosomal recessive MyD88- and IRAK4-deficient humans have been identified. The patients have a predisposition to severe bacterial infections, and *S. aureus* was the cause of invasive infection in 14 and 20% of IRAK4- and MyD88-deficient patients, respectively. The patients also had recurrent, localized skin infections often caused by *S. aureus* (Picard et al., 2011).

The above-mentioned knock-out studies in mice as well as the autosomal recessive MyD88 and IRAK-4 patients demonstrate

that TLR2 and MyD88 are of importance in protecting against *S. aureus*. Epidemiological studies show no or little association between TLR2 R753Q and/or the GT repeat and severe *S. aureus* infection (Lorenz et al., 2000; Moore et al., 2004); however, an association between a TLR2 R753Q polymorphism and nasopharyngeal *S. aureus* carriage among healthy infants was recently found (Vuononvirta et al., 2011). Also, among AD patients, which are often colonized with the bacteria, 9 out of 78 patients (11.5%) carried the TLR2 R753Q variant (Ahmad-Nejad et al., 2004). Only a few studies in mice have addressed the involvement of TLR2, IL-1R, or MyD88 in nasal carriage of *S. aureus*. However, inoculation of *S. aureus* COL into the nasal cavity of TLR2 and TLR4 knock-out mice showed that TLR2 was important for the early innate immune response in the mice, while no significant differences in colony forming units were found between mice 7 days after inoculation (González-Zorn et al., 2005).

Toll-like receptor signaling can also be influenced by ligand-bound glucocorticoid receptor (GR), resulting in reduction in the expression of pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines (Moynagh, 2003). There are multiple isoforms of GR arising from alternative splicing and translational events of the single gene and various polymorphic variants exist (Tait et al., 2008). The exon 9β and ER22/23EK polymorphisms were found to be associated with a 68% decreased and 80% increased risk of *S. aureus* nasal carriage, respectively (van den Akker et al., 2006). The most common glucocorticoid found in human is cortisol. However, no differences were found in the long-term cortisol level among carriers and non-carriers of the bacteria (Manenschijn et al., 2011).

Signaling through NLR

The NLRs are divided into three subfamilies: NALPs, IPAF/NAIP, and NODs. Most NLRs are expressed in the cytosol. NALP1, -2, and -3 are central in creating a PAMP-induced caspase-1 activating complex called the inflammasome, which catalyzes the cleavage of pro-IL-1β and pro-IL-18 into IL-1β and IL-18. The full activation of inflammasomes is tightly regulated and can be divided in two steps. First, it is primed by the TLR-mediated up-regulation of expression of pro-IL-1β and other proteins involved in formation of the inflammasome. A second signal is needed for the assembly of the inflammasome (Martinon et al., 2009; Gross et al., 2011). The formation of NALP3 inflammasome can be induced by for instance *S. aureus* α-hemolysins resulting in IL-1β secretion (Craven et al., 2009).

NOD2 is expressed in skin and in keratinocytes, and recognize peptidoglycan fragments, e.g., muramyl dipeptide, resulting in increased expression of β-defensin 2 and various cytokines (Voss et al., 2006; Siod and Fløisand, 2009). Also, NOD2 has been suggested to play a role in the activation of some types of inflammasomes (Martinon et al., 2009). NOD2-deficient mice are highly susceptible to *S. aureus* infection, and show highly decreased survival after injection with living *S. aureus* (Takeuchi et al., 2000; Deshmukh et al., 2009). Similarly, NOD2-deficient mice subcutaneously injected with *S. aureus* have reduced ability to clear *S. aureus* compared with wild-type mice. The neutrophil recruitment to the skin, as well as serum IgG titers, did not differ between wild-type and NOD2-deficient mice, suggesting that NOD2 is dispensable for normal induction of an adaptive immune response against the bacteria. Instead, NOD2 was required for caspase-1-released IL-1β which induced IL-6 production and increased the neutrophil-mediated killing of *S. aureus* in the skin (Hruz et al., 2009).

NOD2 mutations have been associated with Crohn's disease, and some of the resulting NOD2 proteins were less capable of responding to the bacterial ligand muramyl dipeptide (Turvey and Broide, 2010). Neutrophil dysfunction and increased survival of *S. aureus* have been reported in these patients (Hruz et al., 2009 and references therein). However, even though the NLR pathway is of importance in clearing skin infections caused by *S. aureus*, it remains elusive whether the pathway is of importance in determining nasal carriage status.

The complement system

The main function of the complement system is to promote inflammation, opsonization and lysis of microorganisms, and to remove immune complexes. The complement system consists of a variety of serum proteins that can be activated by microorganisms by three different routes, the classical pathway, the lectin pathway, and the alternative pathway. The classical pathway is activated by C1q binding to antibodies localized on the surface of the bacteria. The lectin pathway is activated when proteins such as MBL attach to the carbohydrate on the bacteria thereby recruiting the MBL-associated serine proteases (MASPs), while the alternative pathway is activated when C3b covalently binds to a microbe. The activation of any of these pathways results in activation of C3, resulting in production of the anaphylatoxin C3a and the opsonin C3b. The assembly of C5 convertases occurs, resulting in the production of anaphylatoxin C5a, as well as C5b and C6–C9, resulting in the production of the lytic membrane-attack complex (MAC) which may lyse the microorganism (Kemper and Atkinson, 2007). The complement system is highly regulated. The regulators of complement activation (RCA) include various proteins, as for example factor H, which acts to destabilize the C3 convertase or promote the factor I-mediated cleavage of C3b into iC3b (Lambris et al., 2008). Another important RCA is the serine protease C1 inhibitor, which regulates both the classical and MBL pathway by inhibiting C1s/C1r and MASP-1/MASP-2, respectively (Zeerleder, 2011).

Mannose-binding lectin is a liver-produced circulating plasma homomultimer complex. Three common polymorphisms are found in exon 1 (R52C, G54D, and G57E), which interfere with the oligomerization ability of the polypeptide. The mutations result in D, B, and C alleles, respectively, while the wild-type allele is named A. The B and C variants occur in 22–28 and 50–60% of Eurasian and sub-Saharan African populations, respectively, while the D variant reaches frequencies of 14% in European populations. In addition, there are two common polymorphisms also identified in the upstream promoter region, which determine serum levels by regulating protein expression. Therefore, the level of functional MBL in serum differs among human population due to a high frequency of various polymorphisms in different combinations. Still, the level is relatively constant within the individual and increases only two- to threefold upon infectious or inflammatory challenge (Turner, 2003; Ip et al., 2009).

Mannose-binding lectin is produced at the nasal mucous lining, and wild-type MBL haplotype A is associated with carriage (van Belkum et al., 2007), while the nasopharyngeal bacterial colonization rates were significantly higher in infants with the variant types B, C, and D of MBL (Vuononvirta et al., 2011). MBL is involved in opsonophagocytosis, and found to interfere with TLR2/TLR6-mediated signaling and expression of cytokines in professional phagocytes (Turner, 2003; Ip et al., 2009), but it is not known whether the variants of MBL differently influence these functions. Another protein that influences complement activation is the acute phase protein C-reactive protein (CRP). Single nucleotide polymorphisms (SNPs) found in CRP include C1184T (rs1130864), C2042T (rs1205), and C2911G (rs3093068). The individual SNPs are not associated with *S. aureus* carriage, but the CRP haplotype 1184C, 2042C, and 2911C were overrepresented in non-carriers in a large cohort (Emonts et al., 2008). A challenge in finding SNPs associated with carriage is the huge heterogeneous genetic background between individuals. One group investigated nasal carriage among 154 adult Wayampi Amerindians living in an isolated village in the Amazonian forest. The SNPs CRP C2042T and C1184 were found to be significantly associated with persistent carriage in this population (Ruimy et al., 2010).

Various bacteria circumvent the complement activation by various mechanisms, including recruiting complement regulators to its surface (Laarman et al., 2010). Recently, *S. aureus* was found to recruit the complement regulator factor H, which via factor I mediated cleavage of C3b, evaded the alternative pathway (Sharp and Cunnion, 2010). Polymorphisms in factor H may influence its expression, or the ability to interact with Sbi or other *S. aureus*-derived proteins. So far, only the Y402H variant of factor H has been tested in epidemiological studies and found not to be associated with nasal carriage of *S. aureus*, but instead with occurrence of boils (Emonts et al., 2008). Another complement regulator is the serine protease C1 inhibitor. An SNP in position 480, replacing a valine with a methionine, has been found to be associated with *S. aureus* carriage, and the V/V genotype was significantly overrepresented in non-carriers (Emonts et al., 2007). Taken together, some association between certain polymorphisms in complement genes and nasal carriage of *S. aureus* have been found, but the molecular mechanisms behind these remains to be investigated.

Professional phagocytes

The presence of *S. aureus* results in increased levels of various AMPs, but also cytokines, chemokines, formylated methionine-containing proteins and complement which all contribute to neutrophil rolling, adhesion, diapedesis, and thereby recruitment of the neutrophils into the skin. The phagocytes engulf the bacteria, and kill them by generating reactive oxygen and nitrogen species, various AMPs, proteinases, and acid hydrolases (Foster, 2005; Miller and Cho, 2011). In addition, neutrophils may try to defend the host against the bacteria by creating extracellular traps, which prevent the microbe from spreading and ensure a high local concentration of antimicrobial agents (Brinkmann et al., 2004).

AIDS patients suffer from impaired phagocytosis of *S. aureus* by granulocytes and monocytes. Defects in neutrophil differentiation, migration, or in ability to create a respiratory burst caused by other diseases might also influence *S. aureus* survival (Bouma et al., 2010; Miller and Cho, 2011). Chronic granulomatous disease patients, for example, have a defect in either of the proteins creating NADPH oxidase complex in phagocytes, and have recurrent bacterial infections which often are caused by *S. aureus* (Miller and Cho, 2011). The nasal secretion among persistent carriers contained the neutrophil specific α-defensins 1–3. Light microscope analysis of Wright-stained nasal fluid confirmed the presence of neutrophils in donor fluid from carrier and acute rhinitis patients, but not in fluid from normal donors (Cole et al., 2001). However, whether these cells had a disturbed ability to clear bacteria was not addressed.

Carriage of S. aureus and adaptive immunity

Several studies have addressed whether human carriers have elevated levels of antibodies against *S. aureus* antigens compared to non-carriers. Many of these studies reveal that carriers have antibodies against various *S. aureus* antigens (Holtfreter et al., 2006; Wertheim et al., 2008; Verkaik et al., 2009a; Colque-Navarro et al., 2010). This may be one of several explanations of why human nasal carriers of *S. aureus* have some degree of protection against fatalities caused by infection (Holtfreter et al., 2006).

One could ask whether the elevated level of antibodies in carriers is caused by nasal colonization as such or by minor *S. aureus* infections due to scratches in the skin, etc. This question was recently addressed, and it was found that even though certain non-enterotoxin superantigens are expressed during nasal colonization, they did not induce systemic neutralizing antibodies. This suggests that nasal colonization of intact epithelium is not sufficient for the induction of a strong serum antibody response (Burian et al., 2012). Similarly, artificial colonization of adult volunteers with a laboratory strain of *S. aureus* did not elicit significant changes in the existing antibody repertoire (Broker and van Belkum, 2011). These studies may suggest that elevated levels of antibodies in carriers are not due to colonization *per se*.

It has also been questioned whether immunization can prevent colonization. Immunization studies in mice or cotton rats using IsdA, IsdH, or ClfB showed that immunization protected or reduced the level of *S. aureus* colonization (Clarke et al., 2006; Schaffer et al., 2006). However, young children with placental transfer of maternal IgG against *S. aureus* antigens were not protected against colonization (Verkaik et al., 2009b; Broker and van Belkum, 2011). More investigations are needed in order to finally state whether immunization can be used to prevent colonization.

Th17 and cutaneous S. aureus

Differentiation of $CD4^+$ T cells yields the T helper cells (Th) Th1-, Th2-, Th17-, and the regulatory T cell (Treg). The signature cytokines of Th17 are IL-17A and IL-17F, but the cells can also produce IL-21 and IL-22. The differentiation of $CD4^+$ T cells into Th17 cells is dependent on cytokines such as IL-1β, IL-6, and IL-23. The presence of these results in activation of JAK–STAT signaling pathway, which in turn stimulates the differentiation and production of the signature cytokines (Minegishi and Karasuyama, 2009; Ochs et al., 2009).

Several patients with hyper-IgE syndrome (HIES), or Job's syndrome, have mutations in *STAT3* thereby resulting in reduced ability of T cells to differentiate into Th17 cells. These patients suffer

from recurrent skin and pulmonary infections, often caused by *S. aureus*. AD and HIV patients are also predisposed to *S. aureus* cutaneous infections, which may be due to decreased Th17 cell responses and/or $CD4^+$ cell counts. Similarly, individuals with autoantibodies against Th17-derived cytokines suffer from *Candida* infections and *S. aureus* skin infections. Studies from mice confirm that IL-17F and IL-17A are involved in host defense against *S. aureus* skin infection (Miller and Cho, 2011). However, whether or how these T cells influence nasal colonization is not known.

STRAIN-DEPENDENT INFLUENCE ON HOST CYTOKINE RESPONSE

The genome of *S. aureus* consists of a conserved core part and variable parts (Witney et al., 2005; McCarthy and Lindsay, 2010). The population structures of the nasal *S. aureus* isolates clearly illustrate diversity that can be clustered in various groups (Melles et al., 2004; Sakwinska et al., 2009; Sangvik et al., 2011). It is also debated whether certain clusters, subclusters, or strains are more virulent or better colonizers than others (Feil et al., 2003; Melles et al., 2004; Lindsay et al., 2006; Muthukrishnan et al., 2011).

A few studies have compared the various *S. aureus* strains in their ability to influence the expression of cytokines and AMPs. Although the molecular mechanisms remain elusive, 10 heat-inactivated clinical isolates varied in their ability to induce β-defensin 1, β-defensin 2, CAP18, and β-defensin 3 in human primary keratinocytes (Midorikawa et al., 2003). Also, comparisons of so-called carrier and non-carrier strains of *S. aureus* showed that they varied in their ability to interfere with expression of TLR2, β-defensin 3, IL-1α, IL-1β, and IL-1Ra in nasal epithelial cell cultures (Quinn and Cole, 2007; Quinn et al., 2009). The strains also varied in their ability to form biofilms, and in sensitivity to IL-1α-mediated growth inhibition (Quinn et al., 2009). Studies in human umbilical vein endothelial cells have shown that the various *S. aureus* strains are equally invasive, while they differed greatly in their ability to induce inflammation perhaps depending on presence/absence of the *agr* (Grundmeier et al., 2010). Another putative mechanism affecting influence on cytokine expression is presence or absence of β-hemolysin in the *S. aureus* strains, as β-hemolysin is shown to influence endothelial IL-8 expression (Tajima et al., 2007, 2009). All these studies indicate that the cellular response may vary depending on the *S. aureus* strain, which may influence the success for colonization of a particular strain.

CONCLUSION

Persistent nasal carriage of *S. aureus* is a complicated interplay between host and bacteria. In order to persistently colonize a human host, *S. aureus* must adhere to the skin and avoid being eliminated due to the human immune response. Presence or expression of appropriate MSCRAMMs and immune evasion molecules are needed for successful colonization. Moreover, the microflora in the nasal cavity influences the ability of *S. aureus* to colonize. The adhesion involves correct combinations and expression levels of bacterial adhesins and corresponding host interaction partners. Persistent carriers may have reduced expression levels of other antimicrobial molecules and/or a less efficient combination of these on the skin. Indeed, lower expression of β-defensin 3 is found to be associated with carriage. Another option is that the persistent carriers have polymorphic variants of the antimicrobial molecules, or enzymes involved in making these, resulting in less efficient molecules or reduced amounts of the antimicrobial molecules, respectively.

Nasal secretions among carriers reveal presence of certain AMPs including the neutrophil-derived HNPs. This may indicate *S. aureus*-mediated activation of the immune system, but without eliminating the bacteria. The molecular mechanisms behind this phenomenon remain to be investigated. Keratinocytes and immune cells also express various PRRs. Activation of these may result in production of antimicrobial molecules and/or production of various cytokines. Polymorphic variants of PRRs may interfere with their ability to detect PAMPs and thereby the host response. Similarly, as activation of PRR may result in activation in signaling pathways resulting in an altered gene response, polymorphic variants in any of these molecules may interfere with the ability of the host to respond to the presence of the bacteria.

Future studies will provide further knowledge regarding host and bacterial determinants involved in colonization. *S. aureus* colonization is an important risk factor for infection. By identifying determinants involved in colonization, putative molecules inhibiting colonization may be produced, which again will lead to reduced risk of infection by the endogenous *S. aureus*.

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