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

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**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, 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 cer-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.

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 cer-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.

**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
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 ( Neale et al., 2009; Harr et al., 2010). The keratinocytes in the basal layer (stratum basale) express the protein filaggrin, which is internalized by scavenging phagocytes (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 (Kisch 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 ( Neale et al., 2009; Harr et al., 2010). The keratinocytes in the basal layer (stratum basale) express the basal keratins K5, K14, and K15. Through continuous proliferations, 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 secretergory 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 cornocytes is called stratum corneum or the corniﬁed layer. Cornocytes 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, glyceryl 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 transpidermal water loss (Keric et al., 2008). The components of natural moisturizing factor include urocanic acid (UCA) and pyrrolidine 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 (Mijalovic 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). CIB 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 corniﬁed envelope proteins loricrin and involucrin are identiﬁed as IsdA interaction partners (Clarke et al., 2006). 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; Corgian 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 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 (pK280:SadC) showed that expression of SaaG reduced S. aureus adherence to fibrinogen and fibrinectin. Thus, certain MSCRAMMs might mask the adhesive functions of other adhesins, and possibly even change the tropism for the host cell (Koche 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 SadD and SdrH (Muthukrishnan et al., 2011).

Cells from different donors provide variable levels of adhesion to S. aureus (Alv 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 K67 (a marker of cell proliferation; Schulein and Gerdes, 2000), involucrin, loricin, filaggrin, and the keratins K5, K6, K10, K16, and K17 (Jensen et al., 2014). Loricin, 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, fibronecctin, 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**

Burián 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 cfb, ida, fnbB, atlA, eap, WTA, genes involved in cell surface dynamics/remodeling (scd, outA, atlA) and immune-modulatory factors (sak, cdp, sip, spa, eap) under these conditions. In contrast, the major toxins (lta, 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 H2O2 tolerance, were found to be associated with nasal carriage in a cotton rat model (Cougnon 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 (Scor 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 (Kismer and Poschel, 2011).

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

The nares are colonized by a temporarily 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., Enterobacteriaceae) (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 acnes (Frank et al., 2010). Bogatov 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. (2009) 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. Lima 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 affects 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 H₂O₂-mediated bacterial interference. Hydrogen peroxide produced by S. pneumoniae kills lysozymic but not non-lysozymic staphylococci by inducing the SOS response. The SOS response induces resident phagocytes and thereby lysis and H₂O₂ lethality (Sélva et al., 2009). It has also been shown that production of H₂O₂ by viridans group streptococci may inhibit MRSA colonization of oral cavities in newborns (Uehara et al., 2001). But in mixed-oculocutaneous 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 H₂O₂ secreting or non-H₂O₂ 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 polymorphism 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., 2005), 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 Kydionius, 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., 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). In the following, we intend to provide a few examples of AMPs and proteins, and to describe their known effects 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 (bHD1–4) 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; Kuich et al., 2007). Keratinocytes have constitutive capacity to kill S. aureus, a phenomenon that is dependent on β-defensin 3 (Kuich 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 (Kuich 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 (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-D3 has been found to enhance the antimicrobial function against S. aureus in vitro (Schauere et al., 2007, 2008). The promoter of the antimicrobial proteins cathelicidin and β-defensin 2 have vitamin D responsive elements (VDREs) (Goembart et al., 2005), and 1,25-(OH)2-D3 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-D3 has been found to induce CD14 and TLR2 expression in keratinocytes (Schauere 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 population (Claisen et al., 2005). There is also reported an association between vitamin D deficiency and MRSA nasal carriage (Mattheson 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 (Bieg 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 (Handstra 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) (Dutarski 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/Grov, CXCL5/MIG, CXCL10/IP-10, CXCL11/IFN-γ, CXCL12/SDF-1, CXCL13/BCA-1, CXCL-14/KRL, CXCL1/Lymphotactin, CCL15-309, CCL18/Ltas, CCL17/TARC, CCL18/PARC, CCL20/MIP3α, CCL21/SLC, CCL22/MDC, CCL25/TECK, IL-1α, and IL-1β (Tang 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; Pynnönen et al., 2011). Carriers of S. aureus have elevated levels of α-defensins 1–3, β-defensin 2, lactoferrin, and slgA 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 secretions 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 (Pynnönen 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 CD14 (Fournier and Philpott, 2005; Nielsen 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 ficolins may also bind to pathogens, thereby activating the complement system followed by opsonization and increased phagocytosis of the bacteria (Casanova and Abd, 2004).

**Signaling through TLR**

Toll-like receptor 1, a transmembrane glycoprotein, which consists of an extracellular domain, a transmembrane domain, and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain. Ligand binding
A simplified overview of how a cell responds to the presence of \textit{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 NF\textsubscript{κ}B. The nuclear localized NF\textsubscript{κ}B stimulates the transcription of AMPs and cytokines (e.g., IL-1\textbeta). 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-1\textbeta processing and secretion. The activation of NOD2 can result in activation of NF\textsubscript{κ}B 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.

Subcutaneous inoculation of \textit{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 \textit{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 \textit{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 \textit{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 \textit{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 (Lorente et al., 2004; Moor, 2004); however, an association between a TLR2 R753Q polymorphism and nasopharyngeal S. aureus carriage among healthy infants was recently found (Vuosonvirta 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 into the nasal cavity of TLR2 and TLR4 knockout 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).

null-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 (Movagh, 2003). There are multiple isoforms of GR arising from alternative splicing and translational events of the single gene and various polymorphic variants exist (Tast et al., 2008). The exon 9 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 (Mannenschin 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 (Crawen et al., 2009).

NOD2 is expressed in skin and in keratinocytes, and recognizes peptidoglycan fragments, e.g., muramyl dipeptide, resulting in increased expression of β-defensin 2 and various cytokines (Voss et al., 2006; Södén and Forsand, 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 (Takesuchi 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 those patients (Heux 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 lyisis 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 (MASP)s, 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 oposin C5b. 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/MA SP-2, respectively (Zerreieder, 2011).

Mannose-binding lectin is a liver-produced circulating plasma homomultimer complex. Three common polymorphisms are found in exon 1 (RS2231240, G57D, 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; Ly et al., 2009).
Mannose-binding lectin is produced at the nasal mucous lining, and wild-type MBL haptotype 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 haptotype 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 (Runyon 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 Sh 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 CI 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/N 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 hydroxides (Porer, 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 (Brenkmann 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 secretions from persistent carriers contain 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 (Cote et al., 2003). 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 Cib 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**

Differentialation 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. Immunization studies in mice or cotton rats using IsdA, IsdH, or CibB showed that immunization protected or reduced the level of S. aureus colonization (Holtfreter et al., 2006).

Several patients with hyper-IgE syndrome (HIES), or Job’s syndrome, have mutations in STAT3 which thereby results in reduced ability of T cells to differentiate into Th17 cells. These patients suffer from recurrent bacterial infections which often are caused by S. aureus (Miller and Cho, 2011). The nasal secretions from persistent carriers contain 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 (Cote et al., 2003). However, whether these cells had a disturbed ability to clear bacteria was not addressed.
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+ T cells. Similarly, individuals with autoantibodies against Th17-derived cytokines suffer from Candida infections and S. aureus skin infections. Studies from mice confirm that IL-17 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 (Wimley et al., 2000; 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; Saltonskis 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; Nathuelsen 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 differ greatly in their ability to induce inflammation perhaps depending on presence/absence of the ahr (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 microbiota in the nasal cavity influences the ability of S. aureus to colonize. The adherence 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 HNP5. 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. Polymeric 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, polymeric 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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