Trends in Microbiology

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Review

Commensal Staphylococci Influence *Staphylococcus aureus* Skin Colonization and Disease

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Commensal organisms that constitute the skin microbiota play a pivotal role in the orchestration of cutaneous homeostasis and immune competence. This balance can be promptly offset by the expansion of the opportunistic pathogen *Staphylococcus aureus*, which is responsible for the majority of bacterial skin infections. *S. aureus* carriage is also known to be a precondition for its transmission and pathogenesis. Recent reports suggest that skin-dwelling coagulase-negative staphylococci (CoNS) can prime the skin immune system to limit the colonization potential of invaders, and they can directly compete through production of antimicrobial molecules or through signaling antagonism. We review recent advances in these CoNS colonization resistance mechanisms, which may serve to aid development of pharmacologic and probiotic intervention strategies to limit *S. aureus* skin colonization and disease.

Highlights

Emerging evidence shows that cutaneous host defense relies upon extensive host/commensal cooperation. Resident commensals serve as the 'true' first line of defense at the skin's surface.

Skin commensals, including coagulase-negative staphylococci (CoNS), fortify cutaneous immune competence by broadening the functional repertoire of the skin's innate and adaptive immune cell networks in a manner that bolsters effector responses against pathogenic microbes, while sparing members of the normal flora.

CoNS can employ a diverse array of tactics that directly counter both the carriage and invasion of the common dermatopathogen *S. aureus*.

The therapeutic efficacy of quorum-sensing inhibition has emerged as a promising paradigm for both pharmacologic and probiotic interventions aimed at mitigating *S. aureus*-induced disease.

Bacterial Colonization of the Skin

The skin is the body's largest and most exposed organ system. Recent estimates of the epithelial surface area, accounting for hair follicles and sweat glands, suggest an expanse as large as 30 m^2 [1]. This vast region is densely populated with microorganisms that interact and compete with each other in order to colonize and survive. Recent efforts to analyze the human skin microbiome have shed light on the most abundant bacteria, eukaryotes, and viruses present in this rich environment. Of the bacteria, the skin is dominated by members of the *Staphylococcus*, *Corynebacterium*, *Streptococcus*, and *Propionibacterium* genera [2–4]. Within the *Staphylococcus* genus, the most common skin commensals are members of the CoNS, with the species *S. epidermidis*, *S. hominis*, *S. haemolyticus*, *S. capitis*, *S. lugdunensis*, and *S. warneri* being the most frequently isolated [4,5]. The CoNS are a large and heterogeneous family of staphylococci. As of 2014, 38 species of CoNS have been identified, and this number is predicted to grow as more human and animal isolates are collected [6]. The skin of healthy individuals is colonized by a mixture of these abundant CoNS, present at different ratios depending on whether the site is dry, moist, or sebaceous.

Staphylococcus represents a broad genus of Gram-positive bacteria that colonize the skin and mucous membranes of humans and most mammals. *S. aureus* is the most problematic pathogen of the genus and is known to cause numerous acute and chronic infections [7,8]. Increasingly, outbreaks of methicillin-resistant *S. aureus* (MRSA), which had traditionally been confined to hospital settings and limited to immunocompromised patients, have emerged in the community and caused pandemic disease in immunocompetent populations [9–11]. An obvious consequence of MRSA's capacity to perpetrate community outbreaks among healthy individuals is the increased population of human reservoirs, which thereby affords greater opportunity for transmission and infection. Furthermore, *S. aureus* asymptomatically colonizes approximately 20–30% of the healthy adult population, most often in the anterior nares (nostrils). This translates to 95 million

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colonized people in the USA alone [12]. Surprisingly, *S. aureus* skin colonization rates are relatively low [13], and abundance levels compared to other bacterial skin colonizers are barely detectable [5]. Despite this, *S. aureus* is responsible for 76% of all skin and soft-tissue infections [14], leading to 500 000 hospital visits and 10 million outpatient visits per year [15].

How can *S. aureus* cause so much skin disease yet be such a poor skin colonizer compared to other CoNS? The prevailing view is that the natural microbiota, like CoNS, protect the skin in part by educating the immune system to limit pathogen colonization [16,17]. It is also increasingly appreciated that the CoNS can directly compete with invading skin pathogens by secreting novel natural products. This overarching concept was collectively termed 'colonization resistance' in recent articles [16,18], a term adopted from the gut microbiota field to describe the inhibition of pathogen colonization [19]. In this review, we outline the skin as a barrier and we define the mechanisms used by commensal bacteria to enhance skin immunity to *S. aureus* opportunistic infection. Lastly, we summarize the various strategies through which CoNS directly compete with *S. aureus* to prevent colonization and disease.

The Skin as a Protective Barrier

As the body's most extensive interface with the outside environment, overall health and homeostasis depend upon the skin's ability to maintain structural and functional integrity as a protective barrier. To be effective, cutaneous barriers must exercise dynamic control over many complex physiological processes (e.g., thermoregulation and tissue regeneration) and insulate vital internal structures from environmental challenges, including the threat of infection. In recent years, it has become clear that restricting the capacity of pathogenic microbes from both productively dwelling upon and penetrating through epithelial surfaces is the result of extensive cooperation between the skin's immune system and the communities of commensal microbes that colonize cutaneous surfaces.

The skin's characteristic acidity, lipid density, and lack of nutrients present a seemingly inhospitable terrain, yet the surface of this tissue represents a vibrant ecosystem teaming with abundant and diverse microorganisms, collectively referred to as the skin microbiota. Beginning at birth, the proliferation of microbiota over the entirety of the skin's surface, including interfollicular appendages (e.g., glands and hair follicles), form a metaorganismal, microbial barrier at the skin's most superficial aspect. 16S RNA data obtained from human skin punch biopsies suggest that a square centimeter of skin harbors a million commensal bacteria; multiplying this value by the 30 m^2 estimate of the combined surface area of the skin's interfollicular and follicular territories, this tissue stages a continual dialogue between the mammalian host and ~300 billion microbes [20,21].

The two principal components of the skin are the epidermis and the underlying dermis (Figure 1). In relation to the skin microbiota, keratinocytes (KCs), which constitute 90–95% of epidermal cellularity, are nearest and most numerous. Arranged in stratified layers according to their differentiation state, KCs are potent purveyors of antimicrobial peptides (AMPs) and cytokine/chemokine signals. As such, these cells are endowed with both direct and indirect means of shaping host–microbe interactions occurring at the skin's surface. Perhaps the most significant way KCs calibrate these encounters is by orchestrating the formation of the stratum corneum (SC), which physically separates the viable layers of the cutaneous epithelium from surface microbes. This waxy and waterproof composite material of flattened corneocytes and KC-derived lipid/granule content interlocks at corneocyte interstices to form a tight mechanical barrier that restricts host–microbial engagement. While the underlying mechanisms are continuing to be unraveled,

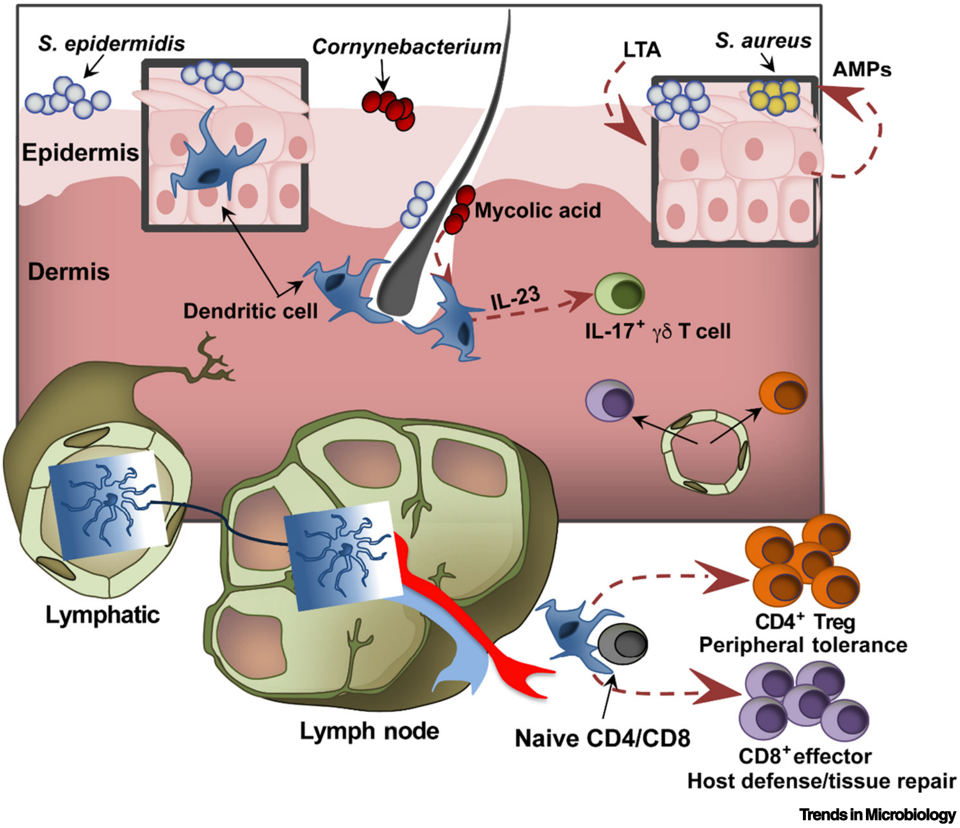


Figure 1. Skin Interactions with Commensal Bacteria. Enriched in the skin's epidermal and dermal compartments, resident dendritic cells (DCs) support cutaneous immune competence by shaping the functional repertoire of the skin's T cell network. In response to encounters with commensal antigens, these skin DCs migrate to the draining lymph nodes and orchestrate the priming of CD4 and CD8 T cells. The T cells fortify the cutaneous immune system through their respective roles in maintaining peripheral tolerance and mediating host immunity and tissue repair. In addition, commensal-derived factors, such as mycolic acid from *Corynebacterium* sp., have been shown to augment specific modes of inflammation via expanding the cellular mediators of the IL-23/IL-17 inflammatory axis. Apart from altering the function of cutaneous T cells, commensal signals such as lipoteichoic acid (LTA) trigger antimicrobial peptide (AMP) production from the skin's predominant cellular constituent, keratinocytes. Overall, the sensitivity of both innate and adaptive elements of the cutaneous immune system to specific commensal cues/signals appears to finely tune both effector and regulatory immune responses in a manner that maintains barrier integrity, encourages commensal occupation, and opposes pathogen invasion.

host and microbial elements can traverse the SC barrier to establish a reciprocal crosstalk under steady-state conditions. As evidence of inside-out movement by the host, Langerhans cells, which are uniformly dispersed throughout the epidermis in a lattice-like arrangement and this region's most abundant antigen-presenting cell population, regularly extend their dendrites into the SC for microbial antigen (Ag) sampling [22,23]. Meanwhile, outside-in movement of microbial components across the SC was revealed by the presence of microbe-derived factors and entire organisms within bona fide dermal territories, which happen to be heavily populated with resident immune cells [24]. That the ongoing crosstalk between cutaneous commensals and resident skin immune cells proceeds in a noninflammatory manner is quite striking. In this way, the homeostatic nature of the interactions between the resident skin immune cells and commensals resembles the analogous dialogue between the immune cells and microbiota of the gut. In marked contrast, experiments with germ-free mice have shown that whereas the development of gut-associated lymphoid tissue is profoundly stunted without microbial exposure, the absence of skin microbiota does not substantially impact the cellular composition of the cutaneous immune system [25]. Rather, the commensal–immune interactions occurring in the skin principally serve to functionally educate and remodel its cellular compartments. However, more comprehensive studies are needed to fully determine commensal–immune interactions in a greater array of immune cell subsets.

Commensal–Skin Interactions Educate Host Immunity

Forged by a coevolutionary process, the nature of the immune–commensal relationship appears to be an overwhelmingly symbiotic one that dictates mutually beneficial outcomes. Considering that the skin and its resident immune cell network continuously engage compositionally diverse and temporally dynamic microbial communities, the challenge associated with experimentally dissecting the immunomodulatory effects of specific microbes is difficult to overstate. Nonetheless, murine models of experimental association of CoNS, as well as other genera of nonpathogenic skin commensals such as *Corynebacterium*, have greatly advanced our understanding of the mechanisms through which the microbiota fortify the immunological functions of the skin. Furthermore, these studies have highlighted the capacity of cutaneous T cells, which populate the skin at an estimated density of 1 million/cm^2, to acquire commensal-specific functional signatures that enable cutaneous barriers to uphold three tasks essential to both the establishment and restoration of tissue homeostasis: (i) sustained immunological tolerance to symbiotic commensals, (ii) robust immunogenicity to invading pathogens, and (iii) efficient tissue restoration/repair following damaging environmental/infectious encounters. Given that the dermatological manifestations of the immune–commensal interactions that are continuously occurring are grossly unremarkable, it seems reasonable to deduce that the majority of these interactions actively support the first task.

Demonstrating the capacity of *S. epidermidis* colonization to shape the skin's CD4$^+$ T cell network in a manner that actively promotes homeostatic tolerance to skin commensals, Scharschmidt *et al*. showed that neonatal exposure to *S. epidermidis* triggers an influx of CD4$^+$ T regulatory cells that promote homeostasis by mediating tolerogenic responses to commensal Ags [26]. Meanwhile, Naik *et al*. showed that the monoassociation of *S. epidermidis* functioned as an immunological adjuvant (Figure 1) by increasing the capacity of cutaneous CD8$^+$ T cells to acquire IL-17 and IFNγ effector functions that translated into heterologous protection against the skin pathogens *Candida albicans* and *Leishmania major* [25]. Demonstrating the capacity of non-CoNS to shape the cutaneous T cell compartment, repeated topical exposure to *Corynebacterium accolens*, or its membranous derivative mycolic acid, results in the expansion of IL-17 producing dermal γδ T cells [27]. Given the importance of IL-17 to host defense against extracellular pathogens, *Corynebacterium* exposure may confer immune prophylactic effects against common microbial pathogens such as *C. albicans* and *S. aureus.* However, a critical balance of IL-17 must be maintained for protective effects, as strong IL-17 polarization is characteristic of pediatric atopic dermatitis [28], and blocking antibodies to IL-17 is associated with better outcomes for psoriasis patients [29]. Furthermore, Nakagawa *et al.* recently demonstrated that IL-17-deficient mice have less severe skin disease pathology scores in the context of *S. aureus* epicutaneous infection than wild-type mice, even though bacterial burdens remain the same [30]. Thus, a definitive pathological or protective role for IL-17 remains elusive.

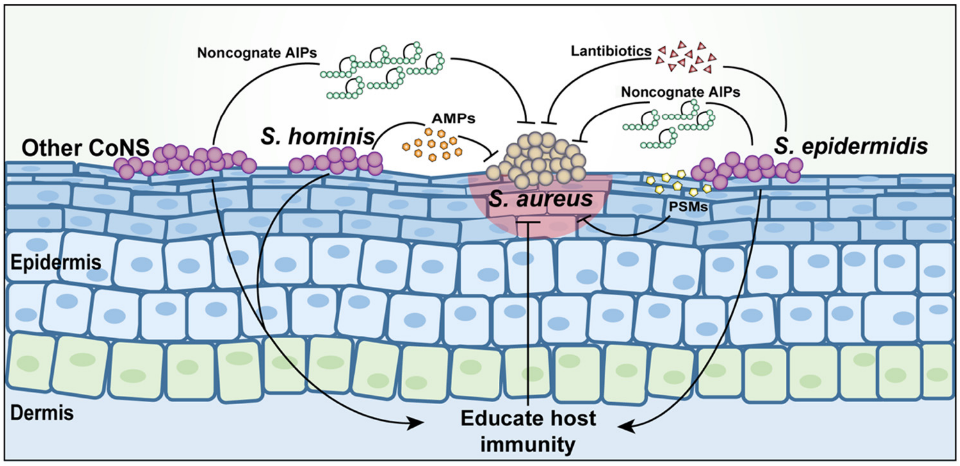
In addition, exciting new findings show that not only do commensal-specific T cell responses endow the cutaneous immune system with the capacity to protect skin from pathogenic invaders, they also impart a repertoire of tissue-repair functions. An implicit intermediary in the dialogue between commensals and the skin's adaptive T cell network are cutaneous dendritic cells (DCs).

These DCs serve as the cellular liaison between the skin, where commensal antigens are concentrated, and draining lymph nodes (LNs), where T cell priming is initiated (Figure 1). Highlighting the ancient origins of this dialogue, studies of *S. epidermidis* colonization show that the expansion of commensal-specific CD8 T cells is restricted to nonclassical MHC-1b (H2-M3) molecules whose emergence pre-dates the evolution of the classical MHC-1a Ag presentation platform. Furthermore, the functional signature imparted by these interactions is impressively pleotropic as it encompasses both heightened immune stimulatory and growth-factor expression profiles, which converge to spur both immunity and cutaneous wound repair [31]. Further underscoring the importance of the microbe/T cell axis in the maintenance of cutaneous barrier integrity, recombination activating gene 1-deficient mice, which lack T lymphocytes, show that cutaneous T cell deficiency corresponds with skin bacteria excessively translocating across and dwelling within the skin and draining LNs respectively [32]. It is important to note that commensals have been shown to promote the immunogenicity and tissue-repair functions of the skin independently of T cells. For instance, a small molecule of <10 kDa secreted from *S. epidermidis* increased expression of human β-defensins (hBDs) in murine skin or human keratinocytes [33]. Additionally, cell-wall components of *S. epidermidis*, such as lipoteichoic acid, engage Toll-like receptor 2 (TLR2) in a manner that restricts excessive inflammatory responses and promotes AMP production from KCs within the contexts of cutaneous wounding and *S. aureus* challenge respectively [33,34]. While cooperative immune–microbe interactions make key contributions to cutaneous homeostasis and host defence, so too do competitive microbe–microbe interactions that abate host exposure to skin pathogens.

Commensal Interactions with Pathogens

When commensals share an ecological niche with pathogenic microbes that can trigger pathophysiological responses, it could threaten the commensal habitat, and the ability to directly compete with the invader could be a beneficial trait. The dominant colonization site for *S. aureus* is the anterior nares [35], and many of the initial direct microbe interaction studies with *S. aureus* have focused on this body site (reviewed in [36]). As some examples, there are reports that artificial implantation of *Corynebacterium* sp. in human nasal cavities can eradicate *S. aureus* [37], and a negative correlation between carriage of *Corynebacterium* and *S. aureus* has been observed in a small cohort of individuals [38]. *S. epidermidis* preadministration in a murine nasal model of colonization prevented subsequent *S. aureus* colonization [39], and *S. epidermidis* strains releasing a serine protease (Esp) cleared *S. aureus* nasal colonization in human volunteers [40]. In follow-up studies, it was revealed that the Esp protease degrades both host and bacterial surface proteins associated with colonization [41,42]. As researchers take a broader sampling of commensal CoNS strains from nasal sites, it is clear that many of them produce antibiotic-like compounds that could aid commensal colonization in a competitive environment [43]. As one prominent example, *S. lugdunensis* was shown to produce a thiazoline-containing cyclic peptide antibiotic, called lugdunin, that inhibits *S. aureus* in the well established cotton rat nasal colonization model [44]. Human nasal carriage of *S. lugdunensis* was negatively correlated with *S. aureus* colonization, though direct competition was not established in this survey [44]. It seems probable that many more of these antibiotic-like compounds derived from CoNS as well as other nasal commensals remain to be discovered.

Similar to the competitive niche of the anterior nares, skin commensals have also devised strategies to deploy their own arsenal of antimicrobials to aid in colonization (Figure 2) [43–45]. For instance, *S. epidermidis* expresses phenol-soluble modulins (PSMs) able to kill both *S. pyogenes* and *S. aureus* [46,47]. Interestingly, *S. epidermidis* also can produce 6-*N*-hydroxyaminopurine (6-HAP), which inhibits GAS growth and also impacts host DNA polymerase and thus inhibits



Trends in Microbiology

Figure 2. The Commensal Skin Microbiota Employ Diverse Strategies to Compete with *Staphylococcus aureus*. Healthy skin is populated by a diverse array of coagulase-negative staphylococci (CoNS) as well as other commensal flora. CoNS compete with *S. aureus* on the skin by producing a wide array of small-molecule products such as antimicrobial peptides (AMPs) and lantibiotics. Another potential mediator of staphylococcal competition is the *agr* quorum-sensing system. While relatively less studied than other small molecules, there is evidence to suggest that interspecies competition exists as shown by the ability of *S. epidermidis* to make an autoinducing peptide (AIP) that inhibits *S. aureus* quorum sensing. We recently published data showing that another rare skin commensal, *Staphylococcus caprae*, also makes an AIP that inhibits all classes of *S. aureus* quorum sensing with nanomolar potency (further described in Figure 4). Thus, noncognate AIPs represent a potential source of competition that merits further investigation in more representative models of skin colonization.

skin neoplasia [48]. Nakatsuji *et al.* recently reported that a *S. hominis* strain produces an AMP that kills *S. aureus* [45]. Transplantation of this strain has therapeutic efficacy for human patients with atopic dermatitis, a dermatopathology characterized by excessive *S. aureus* colonization. Similar to the nasal community, there are increasing examples of skin commensals producing antibiotic-like compounds and many that remain to be identified [45].

Staphylococcal Cell-to-Cell Communication

Extensive interactions occur between commensal bacteria as they compete for resources [49], and new examples of competitive interactions are constantly being uncovered. Not all of these interactions are based on antimicrobials, as signaling crosstalk has been recognized as an effective strategy used by commensals to reduce fitness and slow the growth of a competitor [49–52]. All staphylococci utilize a system of cell-to-cell communication, also called quorum sensing (QS) or the accessory gene regulator (*agr*) system, to coordinate cellular behavior as a function of accumulated cell density [53,54]. The signal sensed by this regulatory mechanism is an autoinducing peptide (AIP), which is a secreted molecule ranging from seven to 12 residues in length, with the last five residues constrained in a cyclic (thio)lactone ring between the terminal carboxylic acid and a cysteine or serine side chain (Figure 3). Though the fundamental scaffold is conserved amongst all known AIPs, the primary amino acid sequence of these molecules can vary widely across staphylococcal species. It remains unclear why the staphylococci evolved a cyclic QS peptide rather than the linear peptide sensed by most other Gram-positives, but it has been demonstrated that the (thio)lactone ring is essential for *agr* binding and activation (reviewed in [54]). The AIP signal is made and sensed using machinery encoded in the *agrBDCA* operon present on the chromosome of all staphylococcal strains. The system functions as follows: AgrD is the peptide precursor

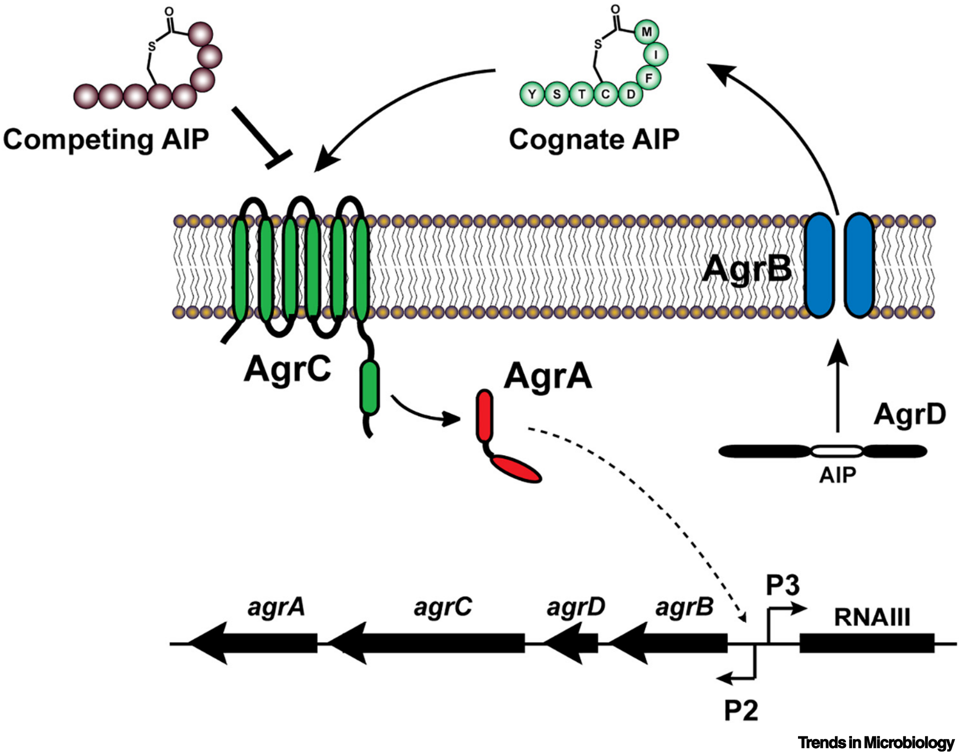
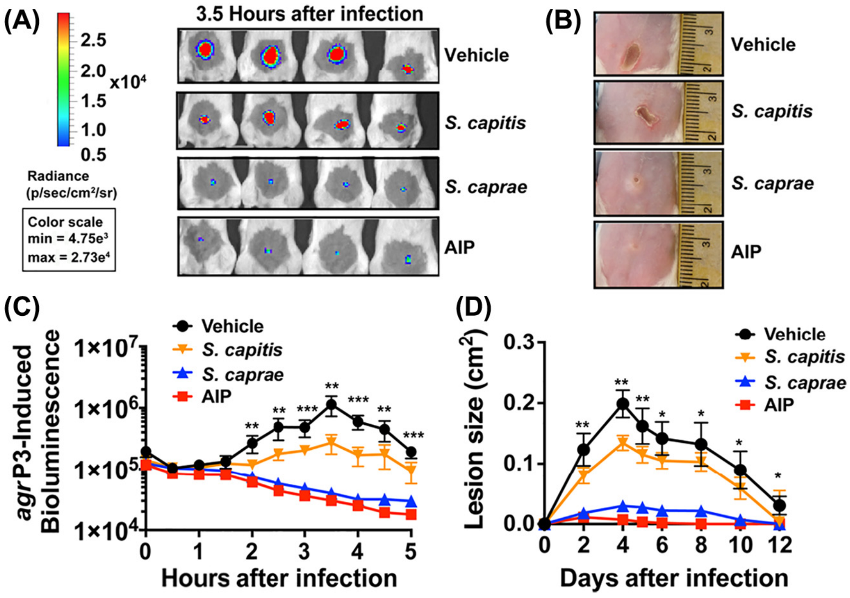


Figure 3. Schematic of the Staphylococcal *agr* Quorum-sensing (QS) System and Signaling Crosstalk. The *agr* locus is conserved in all species of staphylococci, and is composed of the *agrBDCA* four-gene operon divergently transcribed from the RNAIII effector. The AgrB and AgrD proteins build the autoinducing peptide (AIP) signal, and the AgrC histidine kinase is the AIP receptor. When AIP binds to AgrC, phosphoryl transfer to AgrA occurs, inducing the P2 and P3 promoters, resulting in *agrBCDA* and RNAIII expression. RNAIII transcript is the primary effector of the system and upregulates toxins (in *S. aureus*) and exoenzymes (in all staphylococci). The 'cognate AIP' shown is AIP-I from *Staphylococcus aureus agr* type I strains (e.g., USA300), and the 'competing AIP' represents any signal with receptor antagonist activity. These competing AIPs can come from commensal staphylococci. Adapted from [51].

and AgrB is an integral membrane protease involved in generating AIP. As AIP reaches a critical level, it binds and activates the AgrC histidine kinase, which in turn phosphorylates the response regulator AgrA. Activated AgrA binds the P2 promoter to upregulate *agrBDCA* transcription as well as to the P3 promoter to produce an RNAIII transcript. The RNAIII transcript yields a regulatory RNA molecule that acts as the primary effector of the *agr* system by upregulating extracellular virulence factors (e.g., toxins in *S. aureus* or exoenzymes in all staphylococci).

Across the staphylococci, there is divergence within the *agr* locus that results in subtypes within a species, adding complexity to QS interactions. For instance, in *S. aureus* isolates there are four variants of the *agr* locus that result in the production of four distinct AIP signal structures (AIP-I, II, III, and IV), each of which require a unique AgrC receptor for productive signaling. Intraspecies crosstalk (Figure 3) occurs between *S. aureus* strains (e.g., AIP-I inhibiting an *agr* type III strain) in a mechanism termed '*agr* interference' [55], and taking advantage of this mechanism can limit *S. aureus* skin infection [56,57]. Similarly, among *S. epidermidis* isolates there are three types of *agr* system, and crosstalk has been observed between these types [58]. Among the other CoNS strains, there is not yet enough mechanistic information on whether there is variance in the *agr* locus within each species that would lead to intraspecies crosstalk. Furthermore, while the *agr* locus is conserved across all the staphylococci, it is not yet established if all CoNS maintain functional *agr* systems.

The essential role of *agr*-quorum sensing in *S. aureus* skin pathogenesis was elegantly demonstrated in pioneering work by Dr Richard Novick's group. Several notable observations in particular established an unequivocal link between QS and *S. aureus* skin infection [56]. First, the strength of *agr*-P3 during the first few hours of infection is roughly proportional to dermonecrotic injury and therefore serves as a reliable approximation of *S. aureus* virulence factor induction [56,57], and this has now been confirmed in multiple examples [51,59,60]. Second, whereas sterile skin challenge with culture supernatants conditioned by *S. aureus* recapitulated the characteristic dermonecrotic injury, analogous challenges prepared from the *agr* null counterpart failed to induce significant ulceration [56,57]. Finally, disrupting the respective receptor/ligand interactions between AgrC and its cognate AIP by experimentally administering an excess of inhibitory AIP blunts *agr*-P3 activation and manifestations of disease [56,57]. Along these lines, extensive structure/function studies on AIP-based inhibition have led to the development of numerous AIP analogs with *S. aureus* AgrC antagonistic properties (reviewed in [61]). Together, these findings established the central role of *agr* signaling in *S. aureus* skin infection development, and laid the foundation for a new therapeutic paradigm for inhibiting pathogenesis via *agr*-interference [61–63].



Trends in Microbiology

Figure 4. Commensal Competition with Methicillin-resistant *Staphylococcus aureus* (MRSA) during Skin Infection. *Staphylococcus caprae* produces a competing autoinducing peptide (AIP) signal (Figure 2) with nanomolar potency for the MRSA AgrC receptor, completely inhibiting *agr* function. In contrast, a similar skin commensal, *Staphylococcus capitis*, has no ability to produce a competing AIP signal and could not prevent MRSA infection. Briefly: (A) Representative images of *in vivo* bioluminescence induction 3.5 h after challenge with MRSA *agr* P3-Lux +/– 10 μg of *Staphylococcus caprae* AIP, or equal CFU of the CoNS *S. caprae* or *S. capitis.* (B) Representative images show dermonecrosis 5 days after the bacterial challenge. (C) Time course comparison of *in vivo* bioluminescence after intradermal challenge with the indicated conditions. Error bars represent SEM. Post-test *P* value (*) = <0.05, (**) = <0.01, (***) = <0.005. (D) Time course of dermonecrotic lesion size in the indicated challenge conditions. Error bars represent SEM. Post-test *P* value (*) = <0.05, (**) = <0.01. Adapted from our recent report demonstrating that *S. caprae* can compete with MRSA in a skin model of infection [51].

Between staphylococci, interspecies *agr* crosstalk has been examined at only a limited level (Figures 2 and 3). The first well documented example was the *S. epidermidis agr* type I (making AIP-I) inhibiting *S. aureus agr* function [58,64,65]. Recently, the rare skin commensal *S. caprae* [5] was shown to produce an AIP signal that inhibits all *S. aureus agr* types with nanomolar potency, providing a second example of interspecies crosstalk [51]. In direct challenge experiments with MRSA during intradermal skin infection, *S. caprae* provided protection, while a related *S. capitis* strain (without *S. aureus agr* inhibiting activity) showed only minimal benefit (Figure 4). *S. caprae* could also directly compete with MRSA during skin colonization [51], indicating that MRSA requires a functional *agr* system to effectively colonize in a competitive environment. A number of animal CoNS species have been recently identified with some inhibitory action against *S. aureus agr* [66], most notably *Staphylococcus schleiferi*. Increasingly it is also being observed that *agr* crosstalk is not limited to the staphylococci, as *C. accolens* has been shown to release an unknown agent that suppresses quorum-sensing signaling in *S. aureus* [67].

Concluding Remarks and Future Perspectives

Mammalian skin has evolved within the context of perpetual engagement with ambient microbes. In exchange for a stable ecological niche, commensals augment their host's capacity to maintain the robust cutaneous barriers needed for overall health and homeostasis. This partnership is largely a biproduct of the abundance of bacterial mutualists within the microbiome, such as the members of the CoNS. Underlying the host/microbe mutualism that typifies the skin's baseline state, there is a collaborative effort to fortify joint defenses against aggressive pathogens. For their part, commensals convey immunomodulatory signals that bolster both innate and adaptive antimicrobial effector responses. In addition, commensals employ tactics that directly oppose pathogenic outgrowth of invasive microbes, in part due to natural products that are made and released. We are only just beginning to appreciate the chemical diversity on the skin that is the result of commensal growth [16]. As interest in this area grows, efforts will be needed to identify the bioactive small molecules being produced, and different growth media or *ex vivo* models should be tested since laboratory conditions and murine skin models do not match the native skin environment (see Outstanding Questions) [43]. One promising *ex vivo* model is porcine skin, which has been shown to be both immunologically and structurally similar to human skin [68].

Owing to its prevalence and capacity to both dominate and destroy the cutaneous ecosystem, there is perhaps no microbe that poses a greater threat to both the host and skin commensals than *S. aureus*, which is responsible for the majority of bacterial skin infections and figures prominently among the multidrug-resistant pathogens. The lapsing effectiveness of conventional antimicrobial therapies and the rise of community-associated MRSA (CA-MRSA) strains underscores the need for new anti-*S. aureus* interventions and highlights an opportunity to formulate novel modalities that specifically target the invading pathogen while sparing beneficial commensal bystanders. It is important to also consider that *S. aureus* carriage is often a precondition for its transmission and pathogenesis. Recent advances in our understanding of the mechanisms by which *S. aureus* mediates the abrupt transition from a state of benign carriage to invasive infection continue to highlight an indispensable role for QS in pathogenesis (reviewed in [54]). In particular, the *agr* system is critically important for *S. aureus* skin infection, and these phenotypes are amplified in CA-MRSA strains that have higher *agr* activity and produce more toxins and exoenzymes. These properties have made the *agr* system an attractive target of chemical inhibitor development [61–63], and our group and others have identified a number of small-molecule inhibitors that suppress signaling and in turn MRSA infection [69–72]. Considering that every staphylococcal strain has a *agr* system [73] (with varying AIP structures and receptors), it seems possible that the *agr* system has been conserved throughout the species as a colonization determinant, as evidenced by *S. epidermidis* on skin explants [58]. Since the staphylococcal QS system controls production

Outstanding Questions

What are the cellular and molecular mechanisms that commensals exploit to interact with host skin and each other? How do these processes promote cutaneous homeostasis?

What are all the beneficial natural products being produced by skin commensals? Will genome sequencing and improved culturing methods yield new beneficial molecules?

Can we 'predict' *S. aureus* carriage based on our knowledge of skin microbiota? Will our improved understanding of commensal crosstalk shed light on microbiota interactions? Will this understanding also pave the way for the engineering 'super' commensals that can limit dermatopathogen carriage?

Will probiotic interventions aimed at the prevention of *S. aureus* carriage unintentionally upset the ecological balance of beneficial commensals?

of many secreted enzymes that are likely required for growth on host substrates [74], *agr* conservation could be for nutrient acquisition. Additionally, our recent findings with *S. caprae* suggest that QS is also a competition system to gain a fitness advantage in an environment densely populated with other microbes [51].

Moving forward, it is clear that we are only at the early stages of understanding how the skin microbiota interact with each other and the host. Considering how little we know about many skin commensals, it will be increasingly important to understand the mechanisms by which commensals prime skin immune defenses and to identify and characterize the inhibitory natural products they produce. With the dominance of *S. aureus* as a skin pathogen and the elevated *S. aureus* colonization levels during skin dysbiosis [75], understanding how to limit *S. aureus* colonization is an area in need of further investigation to prevent the transition from benign carriage to skin and soft-tissue infections and to mitigate the burden on patients and healthcare systems [76].

Acknowledgments

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