Targeting fundamental pathways to disrupt *Staphylococcus aureus* survival: clinical implications of recent discoveries

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The emergence of community-associated methicillin-resistant *Staphylococcus aureus* during the past decade along with an impending shortage of effective antistaphylococcal antibiotics have fueled impressive advances in our understanding of how *S. aureus* overcomes the host environment to establish infection. Backed by recent technologic advances, studies have uncovered elaborate metabolic, nutritional, and virulence strategies deployed by *S. aureus* to survive the restrictive and hostile environment imposed by the host, leading to a plethora of promising antimicrobial approaches that have potential to remedy the antibiotic resistance crisis. In this Review, we highlight some of the critical and recently elucidated bacterial strategies that are potentially amenable to intervention, discuss their relevance to human diseases, and address the translational challenges posed by current animal models.

Introduction

The progressive increase in the prevalence of antibiotic resistance within circulating strains of *Staphylococcus aureus* is well documented (1). The frequency of resistance to drugs, such as clindamycin, considered to be the mainstays of antistaphylococcal therapy has increased rapidly in recent years (2), and resistance to oritavancin, the most recently US-approved drug to combat methicillin-resistant *S. aureus* (MRSA), has already been reported (3). A clear need exists for the development of novel preventive and therapeutic approaches to combat this pathogen.

The majority of available antistaphylococcal antimicrobial agents exert their bactericidal or bacteriostatic effects by a limited number of mechanisms (Table 1). In fact, all current first-line and second-line therapies (based on expert guidance, refs. 4–6) against *S. aureus*, both MRSA and methicillin-sensitive *S. aureus* (MSSA), exert their antimicrobial effect via one of three mechanisms: disruption of the cell wall and/or cell membrane (β-lactams, glycopeptides, lipopeptides), ribosome-targeted interference of protein synthesis (tetracyclines, macrolides, lincosamides, oxazolidinones), or inhibition of nucleotide production by disrupting folate synthesis (trimethoprim-sulfamethoxazole [TMP-SMX]) (7). In parallel, antibiotic use has selected for *S. aureus* strains with efficient strategies to counteract each mode of attack. A substantial number of important discoveries in the last decade have provided new insights into fundamental mechanisms, such as nutrient acquisition, key metabolic pathways, and evasion of host defenses, that allow *S. aureus* survival in the host. In this Review, we highlight some of these notable discoveries and discuss their clinical relevance and potential implications for developing novel interventions against this important pathogen.

Metabolic and nutritional pathways

The antimicrobial agents TMP and SMX inhibit necessary steps in folate synthesis (8), a key *S. aureus* metabolic pathway. These agents are typically used synergistically and exemplify the treatment potential of targeting *S. aureus* metabolism. Despite the great success of TMP-SMX, which has been used since the 1960s (9), no other *S. aureus* metabolic pathway–disrupting antimicrobial agents are currently approved. However, several recent studies provide promising targets of intervention in this area (Figure 1).

Metal acquisition and competition. A variety of metal ions are essential nutrients for *S. aureus* replication and survival in the host. There is a conflict between metal ion acquisition by the pathogen

Table 1. Targets of intervention against *S. aureus* in current clinical use

Target within *S. aureus*	Commonly used antimicrobial agents	Typical mechanisms of antimicrobial resistance
Cell wall biosynthesis	β-Lactams, glycopeptides	Target modification (e.g., PBP2A); enzymatic inactivation of drug (e.g., penicillinase)
Cell membrane depolarization	Lipopeptides	Mutations in cell membrane target
Protein synthesis (ribosomal inhibition)	Tetracyclines, macrolides, lincosamides, oxazolidinones	Efflux pumps, modification of ribosomal targets
Nucleotide formation (folate antagonism)	TMP-SMX	Mutation in enzymatic target

and sequestration by the host. The ability to overcome host sequestration is critical for staphylococcal survival, and this balance, theoretically, could be perturbed to favor the host. Perhaps the best-studied example of this host-pathogen interaction is the acquisition of iron by *S. aureus* via uptake of host heme proteins, such as hemoglobin. In *S. aureus*, heme uptake is mediated by the iron-regulated surface determinant (Isd) system, which includes a surface receptor for hemoglobin IsdB. Disruption of the Isd system severely attenuates infection in a variety of in vivo models (10–12). Importantly, IsdB represents an example of *S. aureus'* evolution as a human-specific pathogen. IsdB preferentially binds human hemoglobin (13); therefore, the Isd system is a logical target for intervention against *S. aureus*. While active IsdB immunization of patients prior to thoracic surgery was not efficacious in a phase III clinical trial (14), other approaches to manipulate the heme biosynthesis pathway may hold promise. Recently, small-molecule activation of CgoX, a critical heme biosynthesis enzyme, was shown to cause accumulation of coproporphyrin III and render *S. aureus* susceptible to killing by light exposure (photodynamic therapy) in a murine soft-tissue infection model (15), representing the first-known example of photosensitization of *S. aureus* by a small molecule.

The uptake of other cations, such as manganese and zinc, is also essential for *S. aureus* virulence (16). The manganese transporter MntABC is necessary for bacterial growth and resistance to oxidative stress, in part due to the critical role of manganese as a cofactor for superoxide dismutases A and M (SodA, SodM), with SodM being unique to *S. aureus* (17). Diminished manganese uptake renders MntABC mutants highly sensitive to killing by human neutrophils (18) and growth deficient (19). Moreover, MntABC deficiency in invasive clinical isolates similarly renders these strains sensitive to oxidative stress (20). MntC is the manganese-binding surface component of MntABC; it directly competes with host calprotectin, a critical mediator of metal sequestration (21), and vaccination against MntC is protective in a murine model of *S. aureus* bacteremia (22). Given the importance of manganese uptake for *S. aureus* virulence, MntC has recently been included in both passive and active *S. aureus* immunization regimens currently under investigation in mice and humans, respectively (23, 24).

Flexible metabolism in resource-limited conditions. The remarkable ability of *S. aureus* to invade multiple tissues and survive a variety of host stressors is due, in part, to a flexible metabolism. The organism's ability to utilize multiple metabolic substrates is a critical component of pathogenesis, and interference with these pathways represents a potential intervention opportunity. For example, *S. aureus* alters carbohydrate utilization based on host conditions and is well known to facultatively ferment glucose by inducing lactate dehydrogenase in the presence of NO radicals, which are a major component of innate host defense (25). This ability to thrive in the presence of radical NO is critical for pathogenesis and depends on high level L-lactate dehydrogenase activity as well as specific glycolysis substrates. A recent study determined that *S. aureus* depends on glycolysis of hexose sugars, such as glucose and mannose, to survive in the presence of radical NO (26).

The importance of glycolysis for staphylococcal virulence, particularly in light of the discovery of highly efficient glucose transporters on the *S. aureus* surface (27), has potential to be clinically relevant, given the known increased risk of staphylococcal disease in patients with diabetes (28). Glycolysis occurs in all eukaryotes (and is a component of host defense against *S. aureus*, ref. 29); however, a specific inhibitor of *S. aureus* pyruvate kinase, which mediates the final glycolysis step, has been developed (30, 31). As patients with uncontrolled diabetes have increased levels of circulating glucose, specific inhibition of *S. aureus* glycolysis may prevent the pathogen from leveraging this metabolic imbalance, especially if the glycolytic dependence of *S. aureus* occurs to the same degree in humans and mice. However, the contribution of excess glucose
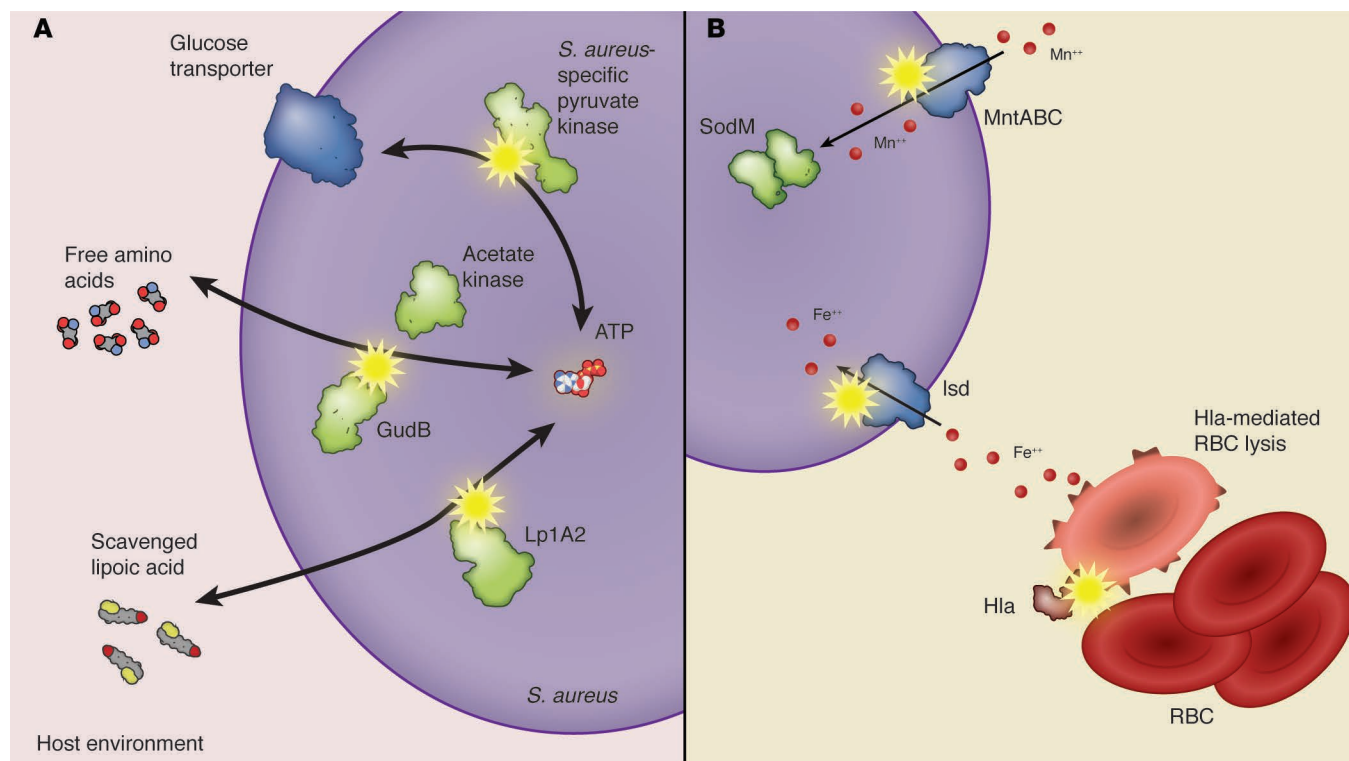
Figure 1. *S. aureus* employs a variety of strategies to ensure adequate nutrition and metabolic function in resource-limited host environments. Recent insights into these pathways have revealed potential targets of intervention, as disruption of nutrient acquisition and energy production may serve to attenuate virulence. (**A**) A summary of metabolic adaptations of interest. *S. aureus* is able to utilize diverse sources of substrates to generate ATP. *S. aureus* produces a glutamate dehydrogenase (GudB) and an acetate kinase that can catabolize free amino acids from the host (32). Additionally, *S. aureus* produces a pyruvate kinase and glucose transporters that facilitate glycolysis (27–31) and is able to scavenge free lipoic acid (33). (**B**) Numerous critical steps have been identified for the acquisition of host metals by *S. aureus*. The MntABC transporter delivers manganese, which, among other roles, is a required cofactor for superoxide dismutase (17–20). α-Hemolysin–mediated (Hla-mediated) RBC lysis liberates host heme, resulting in a bacterial iron source via the Isd system (10–13). Illustrated by Rachel Davidowitz.

warrants further investigation, as other factors, such as compromised vasculature, host cell physiology, and immune compromise, likely contribute to the risk of *S. aureus* disease in patients with diabetes.

Equally important for host tissue invasion and deep-seated abscess formation may be the ability of the *S. aureus* to catabolize amino acids in the absence of available glucose. The glutamate dehydrogenase GudB and an acetate kinase that is capable of generating ATP from amino acids were recently discovered and shown to be necessary for *S. aureus* growth in the absence of glucose (32). Disruption of this pathway likely would not affect colonization or superficial infection but could limit the organism's ability to persist during tissue hemoinvasion. Similarly, *S. aureus* produces an enzyme, Lp1A2, that is not critical for in vitro growth but is required for invasion in vivo (33). This enzyme allows scavenging of lipoic acid, an enzyme complex cofactor critical for intermediary metabolism, in conditions in which free lipoic acid is limited. This serves as another example of how the infection site niche dictates the diverse mechanisms of growth used by *S. aureus*. As focal molecular methods of interference, such as small-molecule inhibitors, improve, these important pathways become increasingly clinically relevant as therapeutic targets of intervention against *S. aureus*.

Evasion and manipulation of host defenses

S. aureus has evolved a wealth of diverse strategies to evade natural host defenses. While *S. aureus* defenses have been well reviewed in recent years, this Review will focus on recent discoveries with high clinical relevance and logical targets for intervention against the pathogen (Figure 2).

Protein A and other cell-surface effectors of immune interference. Numerous explanations for the long list of failed *S. aureus* vaccine attempts throughout the past century have been hypothesized; however, one of the foremost factors may be staphylococcal protein A (SpA). SpA and additional surface-localized factors, such as staphylokinase (Sak), staphylococcal superantigen-like protein 10 (SSL10), and staphylococcal binder of Ig
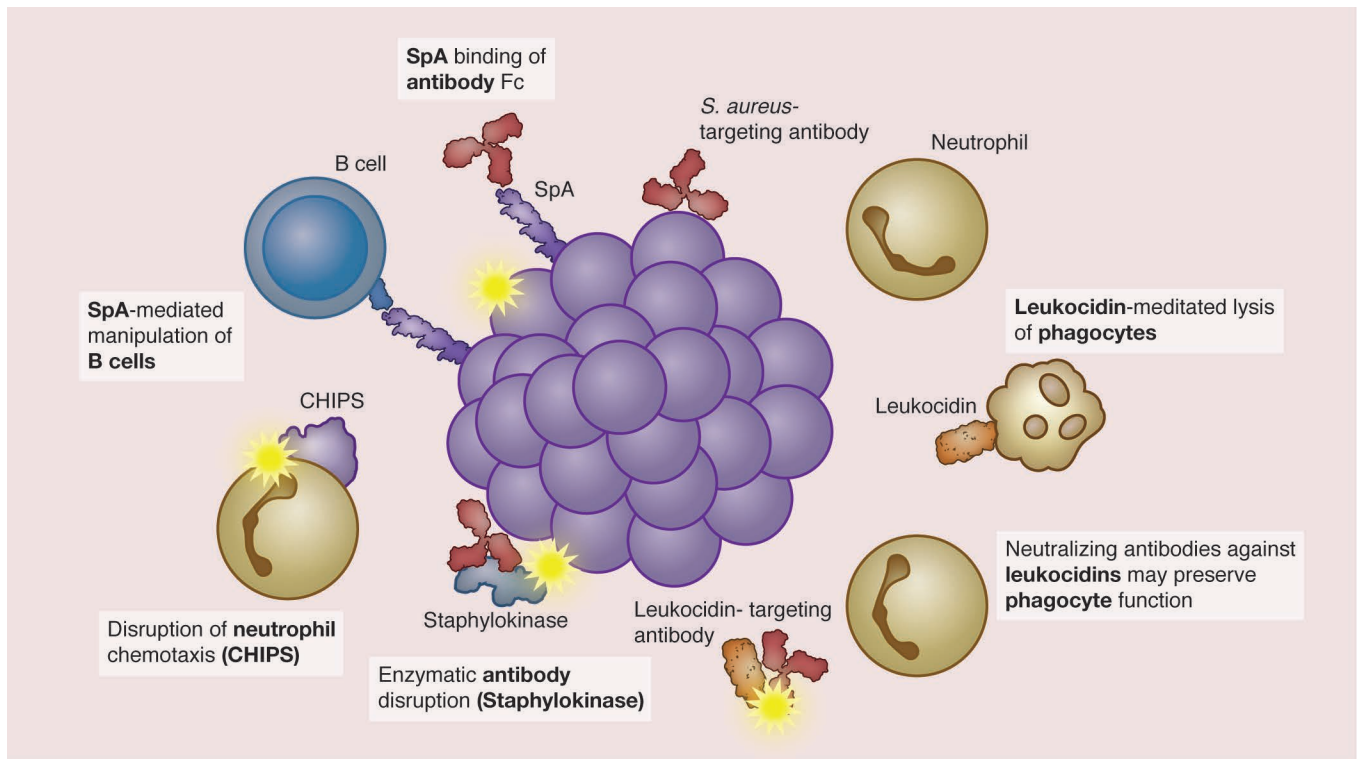
Figure 2. Efficient evasion of both innate and adaptive host defense is paramount to the ability of *S. aureus* to invade and persist in humans. Staphylococcal immune evasion is mediated by several distinct pathways that appear to have high relevance in the setting of human disease and, therefore, represent potential therapeutic or preventive targets. The neutrophil is the primary mediator of innate antistaphylococcal host defense (47), and *S. aureus* expresses numerous proteins capable of interfering with neutrophil function, such as disruption of chemotaxis (CHIPS, refs. 79, 80) or potent lysis (the leukocidins, refs. 59–68) and phenol-soluble modulins (89–94). Human antibodies are capable of neutralizing leukocidin-mediated lysis and represent a potential target for intervention (74–76). Adaptive immunity is disrupted by the pathogen via numerous virulence factors, including staphylokinase (46) and staphylococcal protein A (SpA), which has several known functions, including avid binding of IgG Fc as well as manipulation of B cell responses (34–39). Illustrated by Rachel Davidowitz.

(Sbi), function together to profoundly interfere with antibody binding, complement deposition and activation, and opsonophagocytosis. SpA binds the Fc fragment of human IgG with high affinity, forms a complex with human Fab, and interferes with classical complement activation. More recently, however, the immunologic effects of SpA have been shown to extend beyond interactions at the antibody-effector level. SpA binding to B cell receptors on peripheral murine B cells induces substantial B cell apoptosis in vivo (34), and this apoptotic B cell targeting by SpA is potent and dose dependent (35). In both murine and guinea pig models, WT SpA suppresses B cell responses and leads to clonal apoptosis and prevention of memory responses, both of which are restored during infection with SpA mutant strains (36–38). Finally, SpA is superantigenic, and apparently human B cells are biased toward SpA at the expense of other important antigens, severely limiting host response (39). Despite structural similarities with other staphylococcal superantigens, SSL10 lacks superantigen characteristics. Recently, SSL10 was shown to bind the heavy and light chains of human IgG (40) and to prevent complement component C1q deposition (41), thereby inhibiting classical complement activation. SSL10 appears to be highly conserved across *S. aureus* isolates (42). Sbi is present among diverse *S. aureus* lineages, including clinical isolates (43), and binds human IgG Fc in a Fab-independent manner (44). Sbi appears to also be released from the cell surface, and this form is capable of activating and consuming complement (45). Finally, Sak potently activates human plasminogen on the *S. aureus* surface, leading to cleavage of two critical components of opsonization, IgG and C3b (46). Taken together, these recent data strongly suggest that these anti-antibody factors hinder the typical antibody-antigen interactions that are highly successful for vaccines targeting other bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenza*, and, therefore, conventional vaccine strategies are less likely to be realistic for *S. aureus* disease prevention in humans.

Toxin-mediated targeting of host cells. Neutrophils are critical for human host defense against *S. aureus*, as patients with neutrophil defects have a severe burden of staphylococcal disease (47). It is no coincidence,

then, that *S. aureus* employs a variety of important virulence factors that target host phagocytes, including pore-forming toxins, particularly the bicomponent leukocidins (LukAB [also known as LukGH], Panton-Valentine leukocidin [PVL], LukED) and the γ-hemolysins (HlgAB and HlgCB). The leukocidin family toxins are secreted as monomers, which dimerize and oligomerize on the surface of host cells (particularly phagocytes) to form a pore, resulting in inflammasome activation and cell lysis (48). These toxins exert their effect away from the *S. aureus* surface; therefore, they have potential to be targeted with antibody-based interventions, as the effects of surface factors mentioned above, such as SpA and Sbi, may be at least partially avoided.

α-Hemolysin (Hla) is a pore-forming toxin with a high tropism toward erythrocytes, and, along with LukED and HlgAB, it appears to promote *S. aureus* growth by providing access to host hemoglobin and, therefore, iron (49, 50). Hla is also capable of lysing host endothelial cells and leukocytes, both of which express of the toxin's receptor A disintegrin and metalloproteinase 10 (ADAM10) (51, 52). Hla-mediated endothelial lysis leads to vascular permeability and exacerbation of sepsis in murine models (53), and this permeability may allow *S. aureus* dissemination from the bloodstream into host tissues. The Hla-ADAM10 interaction was recently shown to mediate enhanced *S. aureus* survival in the presence of murine mast cells (54) and to disrupt platelet function and neutrophil signaling (55). Serologic studies indicate that Hla is expressed during human infections, including pneumonia (56) and bacteremia (57), and this complex toxin is a virulence factor being explored for vaccine constructs currently in development (58).

The host receptors for the bicomponent leukocidins have recently been identified (59–62) and appear to have substantial species specificity. For example, LukAB is highly lytic to human phagocytes but only weakly toxic to murine cells due to species-specific differences in the LukAB receptor CD11b (60, 63, 64). Similarly, PVL and HlgCB do not bind murine C5aR1/2, the myeloid receptor for these toxins (65, 66). The species-specific disparity in receptor binding has likely led to an underestimation of the importance of these toxins for human disease. Notably, LukAB, PVL, and HlgCB are moderately toxic to leporine leukocytes, suggesting that rabbits may be a more appropriate model for assessing these human-evolved factors in vivo (63).

Numerous groups have demonstrated that the effects of leukocidins are not limited to pore formation and cell lysis. At sublytic concentrations, LukAB and PVL alter cellular signaling by inflammasome activation, leading to IL-1β secretion (67, 68), and PVL is capable of priming and activating human neutrophils, resulting in enhanced phagocytic function (69). In addition, toxin monomers appear to antagonize their receptors, which may interfere with phagocyte recruitment and function (61, 62). Further, the leukocidins have been identified in noncanonical pairs (e.g., HlgA-LukD), and these noncanonical toxin units can antagonize the cytotoxicity of canonical toxin pairs, suggesting a broader complexity of leukocidin biology than previously appreciated (70–72).

The potential clinical importance of leukocidin family toxins is underscored by their expression during natural human infection. LukAB, in particular, is clearly expressed during invasive human disease, as evidenced by a significant rise in anti-LukAB IgG levels in both recovering children and adults (73, 74) and the purification of human LukAB-neutralizing monoclonal antibodies that recognize diverse epitopes following invasive disease (75). LukAB is also the dominant toxin secreted in vitro under conditions designed to recapitulate the host environment (76–78). These recent findings strongly suggest that the leukocidins warrant continued investigation as potential targets of intervention, particularly for the amelioration or prevention of invasive disease and bloodstream infections.

Phagocyte interference. In addition to lysing host cells, *S. aureus* is capable of disrupting neutrophil function at each step of the innate host response. Chemotaxis inhibitory protein of *S. aureus* (CHIPS) is a prominent factor involved in staphylococcal interference with proper neutrophil migration and was shown to potently interfere with chemoattractants, such as C5a and formylated peptides (79). Importantly, CHIPS exhibits a marked specificity for human neutrophils (80). Similar chemotactic inhibitory functions have since been reported for inhibitors of formyl peptide receptor-like 1, another strong mediator of phagocyte homing (81, 82). SSL5 potently inhibits in vitro chemokine-induced activation of human leukocytes and selectin-dependent neutrophil rolling, thereby adding to the broad armamentarium of antiphagocyte functions of *S. aureus* (83).

S. aureus also employs phenol-soluble modulins (PSMs) for antiphagocyte defense that have proven to be important for virulence in animal models, though the clinical significance of PSMs remains to be determined. PSMs are small peptides that directly target host cells, including phagocytes, and are capable of numerous functions, including host cell activation and cell lysis (89). PSMs are required for virulence in

multiple animal models of *S. aureus* infection, including both noninvasive (cutaneous infection, refs. 89–91) and invasive (osteomyelitis, refs. 92, 93; bacteremia, ref. 89) diseases. PSMs have diverse and complex functions, ranging from cellular recruitment (94) to biofilm support (see below). Notably, all PSMs are exported through a single cassette transporter; therefore, inhibition of this transporter would fully eliminate PSM production (95), supporting this peptide transporter as a point of intervention to curb *S. aureus* pathogenesis.

T cell interference. In addition to interfering with host antibody defense, *S. aureus* deploys multiple strategies to undermine T cell defenses. Expression of staphylococcal superantigens that induce T cell anergy and/or deletion has been well described (96), though it is not fully understood if T cell targeting contributes to human infection, because staphylococcal superantigens exhibit species-specific tropism for human HLA molecules. *S. aureus* also secretes a major histocompatibility complex–like molecule that is thought to drive T cell differentiation toward a Th2 phenotype (97) and toxins that induce T cell cytolysis or apoptosis (59, 98), both of which could adversely affect host protective memory.

S. aureus has recently been shown to undermine development of Th17 cells (99), which may be critical for human defense against *S. aureus* infection, as IL-17–deficient patients with hyper-IgE syndrome have increased susceptibility to *S. aureus* infections (100). Induction of Th17 cells requires expression of Th17-polarizing cytokines by antigen-presenting cells (101); however, *S. aureus* limits release of these cytokines via *O*-acetylation of its cell wall peptidoglycan, which effectively blocks lysozyme-mediated degradation of peptidoglycan (102, 103) and release of embedded proinflammatory pattern recognition molecular patterns (104). By limiting the Th17 memory response, *S. aureus* can effectively reinfect the same host without long-term immunity (99). Patients with *S. aureus* bloodstream infection only mount a modest Th17 response (105). This absence of a robust Th17 response in humans after natural *S. aureus* infection suggests *S. aureus* vaccine would benefit from the inclusion of a Th17 adjuvant (Figure 3).

Adaptations to the host environment

The ability of *S. aureus* to establish infection and persist in a wide variety of host tissues is remarkable. This flexibility is due, in part, to the immune evasion and nutrient acquisition pathways discussed above. Several additional mechanisms have recently been described that detail additional ways that this complex organism manages not only to survive, but also to thrive in resource-limited or toxic host conditions.

Maintaining oxygen balance: antioxidant defenses and virulence despite hypoxia. ROS generated by neutrophils and myeloid cells are a major barrier to successful *S. aureus* infection. ROS are generated upon bacterial phagocytosis by NADPH oxidase and myeloperoxidase and synergize with reactive nitrogen species to induce direct or indirect killing of pathogens. *S. aureus* responds to these insults with an antioxidant defense consisting of ROS sensors, antioxidant molecules, and oxidative repair enzymes. The antioxidant molecules and enzymes commonly expressed upon exposure to ROS are reviewed elsewhere (106). Staphyloxanthin, the eponymous feature of *S. aureus*, is a C30 triterpenoid carotenoid thought to scavenge free radicals with conjugated double bonds (107) and promote resistance to cationic antimicrobial peptide killing by increasing bacterial membrane rigidity (108). Staphyloxanthin enhances *S. aureus* survival in WT mice but not in NADPH oxidase–deficient mice (109, 110), and inhibition of staphyloxanthin biosynthesis reduces *S. aureus* viability in the host, thereby corroborating the antioxidant function of the molecule in pathogenesis (110–112). Moreover, staphyloxanthin inhibition in these studies was achieved via a small-molecule inhibitor, suggesting potential therapeutic application of such a target. *S. aureus* also generates NO, via a NO synthase, as an oxidative product that unexpectedly protects the pathogen against ROS, antimicrobial peptides, and neutrophils (113). NO or downstream metabolites may be protective due to scavenging of HOCL by nitrite, which leads to generation of less ROS (114). Notably, the formation of nitrite downstream of bacterial NO synthesis also appears to contribute to *S. aureus* aerobic respiration by stimulating quinol oxidase, further highlighting the importance of NO synthase (115).

The *S. aureus* antioxidant defense represents an intriguing target for human therapeutics based on the heightened susceptibility of NADPH oxidase–deficient patients to *S. aureus* infections (116). Specific antioxidant targets have been confirmed to contribute to staphylococcal pathogenesis or colonization and include Sod (117), catalase (118, 119), alkyl hydroperoxide reductase (119), and staphyloxanthin (109, 110) (Figure 3). An alternative approach to increase oxidative killing of *S. aureus* is to boost the oxidative function of host phagocytes. In neutrophils, increasing angiotensin-converting enzyme expression has been shown to boost oxidative killing (120). Targeting both bacterial antioxidant defense and boosting host oxidative function represents a potential synergistic approach to combat *S. aureus*.
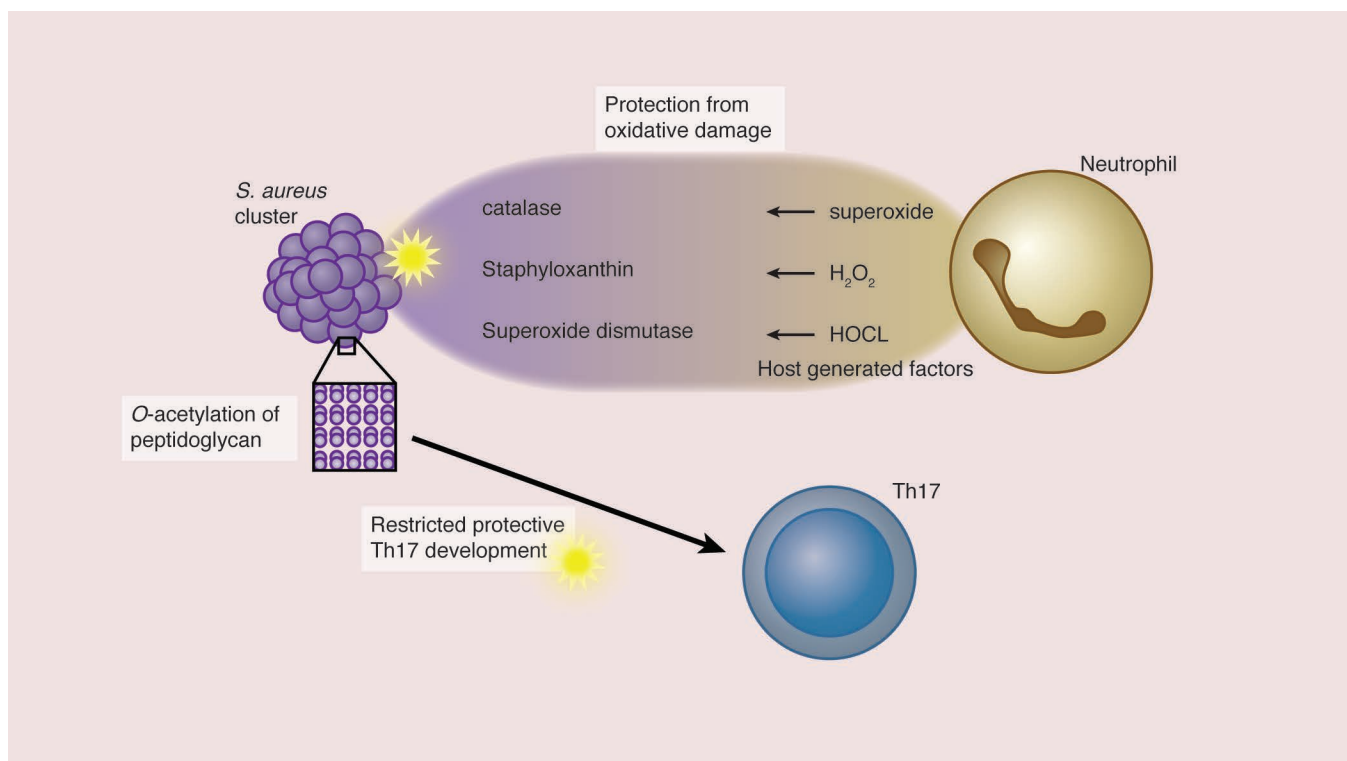
Figure 3. Human immunodeficiency conditions that associate with susceptibility to *S. aureus* infections suggest chinks in the staphylococcal defense that are potentially amenable to intervention. The epidemiology of hyper-IgE syndrome patients suggests that *S. aureus* is susceptible to Th17-mediated killing. Staphylococcal counterstrategies to avoid a Th17 response consist of *O*-acetylation of cell wall peptidoglycan to limit the release of pattern-associated molecular patterns and dampen development of a protective Th17 response (99). Predisposition to *S. aureus* infection in patients with chronic granulomatous disease hints at imperfect staphylococcal protection against ROS in normal hosts. This bacterial weakness could be further exploited by strategies that block staphylococcal antioxidant defenses, such as staphyloxanthin (109, 110), catalase, and superoxide dismutase (117, 118). Illustrated by Rachel Davidowitz.

At the opposite end of the oxygenation spectrum, recent studies have elucidated mechanisms that support *S. aureus* virulence in response to host tissue hypoxia. In a variety of end-organ invasion sites, the organism must maintain virulence in the setting of reduced oxygen tension (121). A recent study used transposon sequence analysis in a robust murine model of osteomyelitis (92) to show that the two-component gene regulatory system SrrAB (122) is critical for staphylococcal survival in hypoxic bone (123). Further, the supernatant from *S. aureus* cultures grown under hypoxic conditions had markedly increased cytotoxicity against a variety of murine and human cells, and SrrAB appears to regulate both hypoxia-induced toxin production and quorum sensing in response to varying oxygen tension. Interestingly, SrrAB overexpression has been shown to repress SpA production and decrease virulence in a rabbit endocarditis model, highlighting the complex relationship between *S. aureus* regulatory systems (124). As both human and murine bones are similarly hypoxic environments (125, 126), these collective observations have high potential clinical importance in understanding the virulence of *S. aureus* in invasive infections, such as osteomyelitis.

Biofilm and persister formation. *S. aureus* employs several additional strategies for survival in a hostile host environment that represent potential opportunities for therapeutic intervention. The biofilm-forming ability of *S. aureus* is well described, particularly as a component of chronic infections (127). The congregation of organisms embedded in an extracellular matrix allows the maintenance of an environment that is relatively impenetrable to standard antibiotic therapies and host defenses. Biofilm formation appears to be under complex genetic regulation, as numerous gene regulatory systems have been implicated in this process (128). Based on murine models, the staphylococcal accessory regulator A (*sarA*) is a promising target for intervening in biofilm production. sarA mutations reduce biofilm viability, allowing enhanced antimicrobial susceptibility, thereby improving in vivo outcomes (129, 130). The quorum-sensing *agr* locus also regulates biofilm maturation (122, 131), and the recent identification of

additional biofilm regulators, such as *xerC* (132) and *rob* (a putative biofilm repressor) (133), suggest that more work is needed to fully understand biofilm regulation before designing fundamental interventions at the genetic level.

Structurally, biofilm formation and maturation require several components that have been recently elucidated and may represent opportunities to prevent or disrupt biofilm formation. A heavily studied component of *S. aureus* biofilm adhesion is poly-*N*-acetylglucosamine (PNAG), also known as polysaccharide intercellular adhesin (PIA). PNAG is a critical adhesin in a variety of in vitro and in vivo biofilm models (134), and human mAbs to this antigen have been shown to exhibit opsonophagocytic activity (135). PNAG is currently being considered as a vaccine target for investigation in humans (136), though it is not produced by some biofilm-forming clinical *S. aureus* strains (137). The PSM peptides (discussed above) also appear to be critical for biofilm maturation, as PSM mutants exhibit deficient biofilm structure and expansion (138, 139).

Antimicrobial therapy also promotes selection of *S. aureus* persister cells, which provide a survival advantage in the presence of antistaphylococcal therapeutics and contribute to chronic or recalcitrant *S. aureus* infections (140). Encouragingly, a relatively new class of antimicrobial agents, the acyldepsipeptides (141), has been shown both to effectively kill *S. aureus* persister cells by activating bacterial proteases and to render persisters susceptible to other antimicrobials (142). The antibiotic resistance profiles of biofilms and persister cells are nearly equivalent, suggesting that biofilms are likely enriched with persister cells (143). This intersection of staphylococcal strategies to survive in the presence of antimicrobials warrants further study and may be a high yield area of potential intervention against the pathogen for a variety of clinically important phenotypes, such as chronic hardware infections, endocarditis, and others.

Manipulation of host coagulation. In addition to providing hemostasis, the coagulation system represents an important component of innate host defense that prevents pathogen dissemination. *S. aureus* has evolved remarkable strategies to hijack the coagulation cascade, allowing the organism to convert this host defense mechanism into a protective fibrin sheath that supports survival and replication (144). This process is primarily mediated by coagulase (Coa) and von Willebrand factor–binding protein (vWbp) (145), both of which bind and activate prothrombin, resulting in thrombin complexes that cleave fibrinogen to generate a bacterial-derived coating of host fibrin (146). Importantly, while Coa and vWbp both activate prothrombin, they are both required for virulence in models of bacteremia and abscess formation, indicating distinct functions in the manipulation of host coagulation (147). The surface component clumping factor A (ClfA) is the major fibrinogen-binding protein of *S. aureus* and also appears to contribute to coagulation manipulation (148). Recent data indicate that all three proteins, Coa, vWbp, and ClfA, are necessary for virulence in animal models of invasive and noninvasive staphylococcal disease (149, 150).

Coa and vWbp activity are important for *S. aureus* virulence in a variety of models, indicating that these factors are worthy targets for potential intervention against the organism. Antibodies against Coa and vWbp are capable of protecting against bacteremia and abscess formation in murine models (147), and a subunit vaccine has shown protective efficacy in mice (151). The ClfA-targeting monoclonal antibody tefibazumab was evaluated as a vaccine candidate in humans and was shown to be safe but had minimal efficacy, despite promising preclinical data (152, 153). Finally, Coa function may also be targeted directly, as antithrombin agents were recently shown to prevent *S. aureus* endocarditis in a rat model, suggesting that thrombin inhibition may be a consideration in high-risk patients with prosthetic heart valves (154).

Current challenges in the translation of basic discoveries to clinical resources

The study of pathogenesis has enhanced the fundamental understanding of how *S. aureus* interacts with the host but ultimately is intended to improve human health. Predictable animal models of disease are paramount for basic discoveries to translate to the clinic. It has long been appreciated that animal models of *S. aureus* infection likely do not exactly recapitulate the human disease, as very high inoculums are needed to induce pathology in mice (155–157). Interestingly, certain phenotypes, such as cutaneous abscess formation, can be produced in mice with relatively similar inoculum size to that required for human infection. However, a notably larger inoculum is required to generate more immune-driven phenotypes, such as shock, in animal models. This discrepancy is likely related to increased interactions at the molecular level between humans and *S. aureus* compared with mice and *S. aureus*.

Animal models have also come under increasing scrutiny in the past decade due to their failure to resolve important *S. aureus* clinical problems. In particular, in emerging community-associated MRSA

strains, PVL expression strongly correlates with necrotizing human diseases but is not required for pathologic phenotypes in mice (158). The long-standing controversy over the role of PVL in disease was unraveled after the discovery that PVL binds strongly to human, but not mouse, C5aR (66). The failed clinical trials of *S. aureus* component vaccines that were successfully developed in mice have also been unsettling (14) and have raised serious concerns regarding key differences between human and mouse immune responses to *S. aureus*.

Although *S. aureus* pathogenesis in mice has been increasingly well delineated, it is unclear what aspects and to what extent murine infections are relevant to human disease. High infectious inoculum is required in most animal models to induce *S. aureus* infection and could clearly alter quorum sensing and the physiologic context under which individual bacterial factors are expressed and studied for functional significance. More importantly, the concept of what defines a human-like disease phenotype remains unclear. In spite of these challenges, mouse models continue to be attractive because of the relative low cost and plethora of available tools to study complex mechanisms.

As a complement to standard mouse models, investigators have had some success modeling specific aspects of pathogenesis in other animals, such as rabbits and rats. The use of other species has not circumvented the need for high inoculums but has, in certain instances, enhanced the modeling of host-pathogen interactions, as was exemplified by evaluation of PVL in the rabbit model (159). Additionally, mice that transgenically express select human receptors provide an alternative approach for studying virulence factors with human tropism (13); however, this approach requires prior knowledge of cognate receptors for the bacterial product.

More recently, several reports have addressed modeling *S. aureus* infections in so-called "humanized" mice (160–162), which are immunocompromised animals engineered to accept human hematopoietic stem cells and subsequently develop a human immune system (163). Initial reports indicate that humanized mice in the NOD/SCID γ (NSG) background may be an improved model, as a reduced inoculum (up to 1–2 logs lower than that required for WT mice in the soft-tissue model) is able to induce dermonecrosis. Moreover, humanized NSG mice exhibit a pathologic phenotype for factors with selective human tropism (160–162). However, the high cost; the potential confounding by nonimmune compartments, such as epithelial cells; and the incomplete humanization of the immune system, including low neutrophil counts and lack of complement, remain barriers for general acceptance of the model.

In the long term, humanized mice with extensive knockin of human genes will likely become available and more faithfully model human infection. Unfortunately, there is no timeline for when these tools may become available; therefore, more immediately feasible steps need to be taken to address the current translational dilemma. For instance, given the importance of neutrophils in *S. aureus* pathogenesis, generation of a humanized mouse with increased numbers of human-like neutrophils, such as by transgenic expression of human G-CSF, will be a major step forward. For each potential model, critical evaluation of the similarity to

Table 2. Animal models of *S. aureus* infection

Animal model	Description	Pro	Con	Remedies
Mice	Most commonly used model	Well characterized; abundant tools; low cost	Require high inoculums; unclear tropism issues	Understanding differences in human and murine responses to *S. aureus*
Other animals	Rats, rabbits, cows	More human-like than mice for select virulence studies	Few tools; require high inoculums; tropism issues	Same as in mice
Transgenic mice	Mice that express human immune or nonimmune proteins	Addresses select tropism problems; benefits of standard mouse model	High inoculums; requires prior knowledge of human receptor	Knockin all relevant immune genes
Humanized mice	Immunocompromised mice engrafted with human HSCs; several models supporting different levels and types of immune cell engraftment	Inoculums potentially closer to human infections, depending on model readout; overcome some human tropism problems	Low human neutrophil count; expensive; unclear compatibility between human and mouse immune components	Evolving next-generation humanized mice; knockin h-GCSF to address major neutrophil issue in modeling of *S. aureus* infections

h-GCSF, human granulocyte CSF.

human disease and possible limitations will be required, as undertaken in a recent review (164) and summarized in Table 2. More direct efforts to understand *S. aureus*–human host interactions and to identify differences between human and murine immune responses to *S. aureus* may further enhance and clarify the clinical relevance of research done in mice. A refined knowledge of the types of human T cell and antibody responses that are protective against *S. aureus* infection will be critical for the development of an effective human vaccine. While addressing these issues will be difficult and may require the generation of new tools, these are challenges well worth pursing to advance our understanding of *S. aureus* disease in humans.

Conclusions and future perspectives

There has been a significant expansion of *S. aureus* pathogenesis research in the past decade that has been fueled by the emergence of community-associated MRSA and increased antibiotic resistance. *S. aureus* metabolism, a once dormant subject, has become an active area of investigation driven by technologic advances, and many *S. aureus* pathogenic mechanisms, such as toxin-related virulence, are now understood at the level of molecular interaction with host receptors. Together, these advances have identified a plethora of potential human therapeutics, but the greater challenge in the coming years will be to validate the relevance of these targets for human diseases; to demonstrate that some of the targets, at least one or two, can be developed into cost-effective reagents as alternatives and/or adjuncts to antibiotics for treating *S. aureus*–infected patients; and to improve basic models of *S. aureus* disease for better translational value.

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