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Staphylococcus aureus, Antibiotic Resistance, and the Interaction with Human Neutrophils

Viktoria Rungelrath and Frank R. DeLeo

Abstract

Significance: *Staphylococcus aureus* is among the leading causes of bacterial infections worldwide. The high burden of *S. aureus* among human and animal hosts, which includes asymptomatic carriage and infection, is coupled with a notorious ability of the microbe to become resistant to antibiotics. Notably, *S. aureus* has the ability to produce molecules that promote evasion of host defense, including the ability to avoid killing by neutrophils.

Recent Advances: Significant progress has been made to better understand *S. aureus*–host interactions. These discoveries include elucidation of the role played by numerous *S. aureus* virulence molecules during infection. Based on putative functions, a number of these virulence molecules, including *S. aureus* alpha-hemolysin and protein A, have been identified as therapeutic targets. Although it has not been possible to develop a vaccine that can prevent *S. aureus* infections, monoclonal antibodies specific for *S. aureus* virulence molecules have the potential to moderate the severity of disease.

Critical Issues: Therapeutic options for treatment of methicillin-resistant *S. aureus* (MRSA) are limited, and the microbe typically develops resistance to new antibiotics. New prophylactics and/or therapeutics are needed.

Future Directions: Research that promotes an enhanced understanding of *S. aureus*–host interaction is an important step toward developing new therapeutic approaches directed to moderate disease severity and facilitate treatment of infection. This research effort includes studies that enhance our view of the interaction of *S. aureus* with human neutrophils. *Antioxid. Redox Signal*. 34, 452–470.

Keywords: *Staphylococcus aureus*, MRSA, CA-MRSA, antibiotic resistance, neutrophil, PMN

Introduction

ANTIBIOTIC RESISTANCE IN BACTERIA is a serious problem globally. According to the 2019 AR Threats Report published by the Centers for Disease Control and Prevention (CDC, Atlanta, GA), 2.8 million people in the United States acquire an antibiotic-resistant infection annually, and there are an estimated 35,000 associated deaths. The problem is similar in other parts of the world. For example, in Europe, there are an estimated 33,000 annual deaths attributed to antimicrobial resistant infections (21). In addition to the reported morbidity and mortality, antibiotic resistant bacteria cause a significant economic burden. For instance, the annual cost burden of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in the United States was estimated to be as great as $13.8 billion (97).

S. aureus resistance to penicillin was reported in the 1940s, and infections with antibiotic resistant *S. aureus* remain a problem today (81, 166). Indeed, MRSA is currently classified as a ''serious threat'' by the CDC, and threat level is based on seven factors including clinical impact, incidence, transmissibility, and available treatments. Consistent with this assessment, *S. aureus* is one of the six ''ESCAPE bugs''—those that cause the majority of nosocomial infections in the United States and are resistant to antibiotics (16). Inasmuch as *S. aureus* has remained a threat to human health throughout recorded

Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA.

history, and because the microbe can readily develop resistance to antibiotics, *S. aureus* will be considered as a model organism for the purpose of this review.

S. aureus is a Commensal Microbe and an Opportunistic Pathogen

S. aureus is a gram-positive, catalase-positive, facultative anaerobic bacterium that colonizes healthy humans and animals. The microbe is also an important opportunistic pathogen. *S. aureus* was first identified in pus from a leg abscess in the late 19th century by Alexander Ogston (122). The bacterium is named for the grape-like appearance of multiple cocci under the microscope and for the golden color of colonies on solid media (*staphyle* is Greek for grape and *aureus* is Latin for golden) (Fig. 1). Approximately 30% of people are asymptomatically colonized by *S. aureus* in the nares and another third are at least transiently colonized (57). In recent years, studies have investigated the interaction of *S. aureus* with the nasal microbiota and found that certain nasal commensal microbes enhance colonization with *S. aureus*, whereas others inhibit colonization (91). This knowledge can potentially be exploited for the development of novel decolonization strategies that are employed to decrease the burden of *S. aureus* carriage in targeted populations (120).

Recent studies have demonstrated that *S. aureus* colonization of humans is more extensive than previously known. In addition to the nose, *S. aureus* can be isolated from many other body sites, including the oropharynx, axillae, groin area, perineum, rectum, and intestine (2, 115). Asymptomatic colonization with *S. aureus* is a risk factor for subsequent infection with the colonizing strain (71, 183). However, infections in colonized individuals are typically less severe compared with those in individuals who are not colonized with *S. aureus*, perhaps because such individuals have developed some protective immunity against severe infection. Other risk factors for *S. aureus* infection include damaged skin or mucous membranes, immunosuppression (*e.g.*, due to chemotherapy, age, congenital neutrophil disorders, or acquired immunodeficiency syndrome), the presence of catheters or prosthetics, and underlying diseases such as cancer or diabetes (106, 178). *S. aureus* is transmitted by direct skin-to-skin contact or *via* contaminated fomites. Thus, *S. aureus* carriers play an important epidemiological role in transmission and perpetuation of disease.

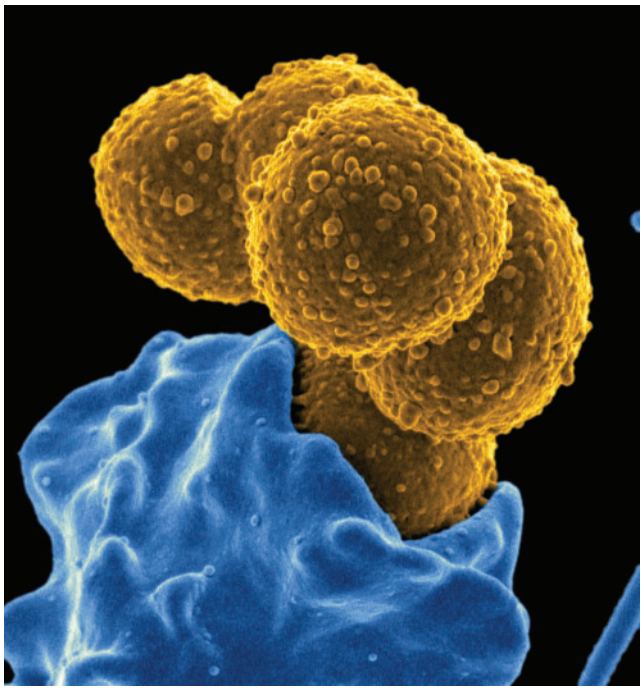

FIG. 1. ***Staphylococcus aureus***. Scanning electron micrograph of *S. aureus* (*yellow*) bound to a human neutrophil (*blue*).

Manifestations of S. aureus *infection*

S. aureus infections can be mild to life-threatening, localized or disseminated, and affect any organ of the body (178). Thus, symptoms and pathologies caused by *S. aureus* are manifold. The most common presentations of *S. aureus* infection are skin and soft tissue infections (SSTIs), bacteremia, endocarditis, pneumonia, device- or prosthetic-related infections, and osteoarticular infections (33, 106, 178). Here, we highlight a selected number of infections or syndromes for which *S. aureus* is notorious.

Moran *et al.* reported that MRSA—primarily USA300 strains—accounted for 59% of all purulent SSTIs reporting to emergency departments in the United States in 2004 (116). These data provided strong support to the idea that *S. aureus* is the leading cause of community bacterial infections. Talan *et al.* made similar findings for 2008, which indicated the high incidence of CA-MRSA had remained unchanged (174). Although *S. aureus* remains the leading cause of SSTIs in the United States, recent data indicate the incidence of SSTIs in emergency departments decreased from 2009 to 2014 (117). These findings are consistent with the reported trends for decreased MRSA SSTIs in this setting (94). Collectively, these and other reports suggest the epidemic of CA-MRSA infections peaked before 2010.

S. aureus is also a leading cause of bloodstream infections (BSIs). For instance, recent multicenter studies reported that *S. aureus* is the most abundant cause of health care-associated BSIs (4, 87). In 75% of cases, *S. aureus* BSI results from a primary infection such as SSTI, pneumonia, or an infected indwelling catheter, whereas in 25% of cases, the primary source of infection remains unknown (178). Other known risk factors for *S. aureus* BSI include human immunodeficiency virus infection, hemodialysis, cancer, recent organ transplantation, and injection drug use (95, 175). Bacteremia caused by *S. aureus* is typically associated with a relatively high mortality rate, which is varied and depends on multiple factors including patient age and comorbidities (178).

S. aureus is the most abundant cause of infective endocarditis in the industrialized world. Such infections represent more than ~25% of all infective endocarditis cases (178), and they are most often health care-associated (49, 178). For example, during the time period of 1998–2009, 28.7% of infective endocarditis cases in the United States were caused by *S. aureus* (during the same time frame, 24.7% were caused by streptococci, which ranked second) (15). Notably, there was a significant increase in the percentage of *S. aureus* infective endocarditis cases during this time period (15). Bor *et al.* suggested that the noted increase in cases is linked to an increase in cardiac devices and patient implants. Consistent

with this idea, patients with prosthetic heart valves and/or *S. aureus* bacteremia are known to be at risk for infective endocarditis (43). *S. aureus* infective endocarditis has an overall mortality rate of 22%–66%, which is greater than that for other pathogens (178).

Pneumonia is an important manifestation of *S. aureus* infection, and *S. aureus* pneumonia occurs in both community and health care settings (178). For example, in a large retrospective cohort study, Kollef *et al.* reported that *S. aureus* is the leading cause (among bacterial pathogens) of health care-associated, hospital acquired, and ventilator-associated pneumonias in the United States (86). MRSA accounted for ~34%–57% of these *S. aureus* pneumonia cases (86). Magill *et al.* reported similar findings for a study that surveyed 199 hospitals in 2015 (108). Mortality rates for health care-associated and hospital-acquired pneumonias have been reported as ~18%–19%, thereby underscoring the importance of *S. aureus* as a cause of severe respiratory infections.

The incidence of *S. aureus* community-acquired pneumonia is less than that of health care-associated or hospital-acquired pneumonias (87), although it can also be severe and/or fatal (50, 62). A characteristic difference between health care-associated or hospital-acquired pneumonias and community-associated/acquired pneumonias is the lack of patient comorbidities in the latter group. That said, CA-MRSA pneumonia-related fatalities have often been associated with antecedent influenza virus infection (53, 62). There is also compelling evidence that *S. aureus* contributed to (or was the underlying cause of) severe or fatal pneumonia during influenza A virus pandemics that occurred in the 20th century (27, 143, 156).

Inasmuch as *S. aureus* is a leading cause of BSIs, it is not surprising that it causes bone and joint infections. Indeed, the organism is the most frequently isolated agent from individuals with infectious osteomyelitis and prosthetic joint infection in multiple regions of the world (89, 138, 145, 178). *S. aureus* can disseminate to bones and joints *via* the bloodstream or direct placement of contaminated prosthetic devices (178). Osteomyelitis is often difficult to treat and can become chronic (138). *S. aureus* can form a biofilm within the bone, (123), rendering the organism less susceptible to host defenses and antibiotics. In addition, *S. aureus* can form small colony variants (SCVs), which are phenotypically characterized by reduced metabolic activity and slow growth (and thus form small colonies on agar plates) and are more resistant to killing than non-SCV *S. aureus* (180). SCVs have been implicated in the pathogenesis of chronic osteomyelitis, thereby contributing to the difficulty with treatment (77).

History and Mechanisms of Antibiotic Resistance in *S. aureus*

S. aureus was susceptible to most clinically useful antibiotics before the modern antibiotic era. Indeed, penicillin was used widely and successfully to treat *S. aureus* (and other) infections in the 1940s and 1950s. Such widespread use of this important antibiotic led ultimately to the development of resistance among many bacterial pathogens including *S. aureus* (81). Kirby discovered that *S. aureus* resistance to penicillin was due to the activity of a penicillinase that he isolated from resistant clinical isolates (81). This penicillinase, now more commonly known as a type of β-lactamase, is an enzyme encoded by the *blaZ* gene, which in *S. aureus* is often located on a plasmid within a transposon (118). Penicillinase hydrolyzes the amide bond of the β-lactam ring of penicillin and ampicillin (126) (Fig. 2A). Penicillinase production is varied among *S. aureus* strains, but those with high production are also more likely to be resistant to other antibiotics, such as tetracycline and streptomycin (142).

S. aureus strains can be naturally resistant to penicillin, or they can acquire resistance during penicillin treatment, as was demonstrated by Spink and Ferris in 1947 (165). Penicillin-resistant *S. aureus* was first isolated in hospitals but did not remain constrained to the health care setting and ultimately caused infections in the community. These infections were largely caused by strains belonging to the phage type 80/81, which was later characterized as clonal complex 30 (CC30) by molecular typing methods (144, 151). The phage-type 80/81 strain was particularly virulent and transmissible, and ultimately became pandemic (144).

Inasmuch as the majority of phage-type 80/81 clinical isolates were penicillin resistant, a new therapeutic was needed to treat *S. aureus* infections. To that end, methicillin

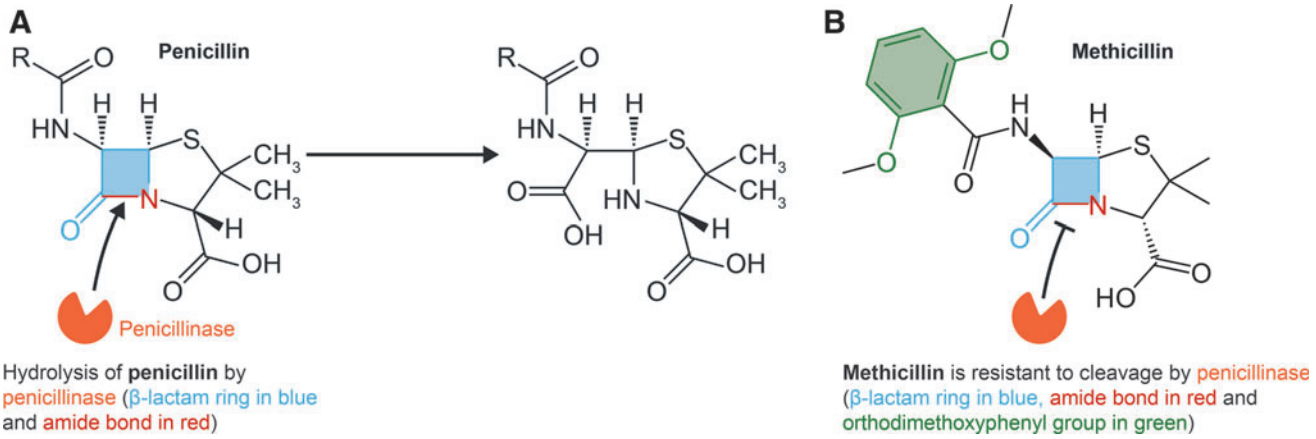

FIG. 2. Interaction of *S. aureus* penicillinase with penicillin and methicillin. Penicillinase hydrolyzes the amide bond (highlighted in *red*) of the β-lactam ring of penicillin and ampicillin **(A)**. Methicillin is resistant to cleavage by *S. aureus* penicillinase (a β-lactamase) due to the presence of an ortho-dimethoxyphenyl group (highlighted in *green*) that sterically hinders the enzyme from hydrolyzing its target amide bond **(B)**.

(marketed as Celbenin [BRL 1241] by Beecham Research Laboratories, Inc.—now GlaxoSmithKline), a semisynthetic β-lactam antibiotic, was introduced in 1959/1960 as a treatment for infections caused by penicillin-resistant staphylococci (82). Methicillin is resistant to cleavage by *S. aureus* β-lactamase due to the presence of an ortho-dimethoxyphenyl group that sterically hinders the enzyme from hydrolyzing its target amide bond (168) (Fig. 2B). Within a year after the introduction of methicillin, MRSA were isolated from hospitalized patients (8, 73).

Methicillin resistance is conferred by the *mecA* gene, which is located on a mobile genetic element known as staphylococcal cassette chromosome *mec* (96). As such, the *mecA* gene is acquired *via* horizontal gene transfer. The *mecA* gene encodes a transpeptidase known as PBP2a, an altered penicillin binding protein that has low affinity for β-lactam antibiotics (23, 46). The reduced binding of PBP2a with β-lactam antibiotics circumvents the ability of these antibiotics to inhibit cell wall synthesis (Fig. 3). Importantly, PBP2a confers resistance to all β-lactam antibiotics—not just methicillin (23). A recent study by Harkins *et al.* suggested that MRSA was present before the use of methicillin as a therapeutic agent (64). This finding changed the long-standing notion that clinical use of methicillin was the underlying factor in the rapid emergence of MRSA. Rather, Harkin *et al.* proposed that it was the widespread use of penicillin that selected for *mecA*-positive *S. aureus* in the 1940s (64).

MRSA epidemiology and subtypes

MRSA has remained a significant problem globally since the 1960s. The pathogen is endemic in hospitals worldwide and is often the leading cause of infections in this setting. CA-MRSA was epidemic in the United States and in other regions of the world in the first decade of the 21st century (24). More recently, the CDC estimated that there were 323,700 cases of MRSA in hospitalized patients in the United States in 2017, and these cases resulted in an estimated 10,600 deaths. The overall prevalence of MRSA among *S. aureus* isolates in 11 countries representing Central America and South America was reported as ~48% for 2004–2007 (149). These findings are consistent with a subsequent study by Reyes *et al.*, who reported that MRSA comprised 41% of all *S. aureus* isolates from 32 tertiary hospitals in Columbia, Ecuador, Peru, and Venezuela (139). In Europe, the prevalence of MRSA-invasive infection differs by country and ranges widely (*e.g.*, the occurrence is 0.9% in the Netherlands and 56% in Romania). As of 2014, Scandinavian countries had the lowest rates of invasive MRSA infections, whereas southern and southeastern European countries have the highest rates of such infections (greater than 25% occurrence).

The sustained high prevalence of MRSA, coupled with the emergence of CA-MRSA, led to the implementation of more robust infection control and prevention measures, and an increased awareness of the scope of the problem (196). Consistent with the implementation of these measures, the number of estimated hospital MRSA infections in the United States has decreased steadily since 2005 (88). Kourtis *et al.* used data that were collected from 400 acute care hospitals participating in the CDCs Emerging Infections Program population surveillance study to estimate MRSA BSI rates in the United States over an 11-year period (88). Notably, there were dramatic reductions in hospital-onset and community-

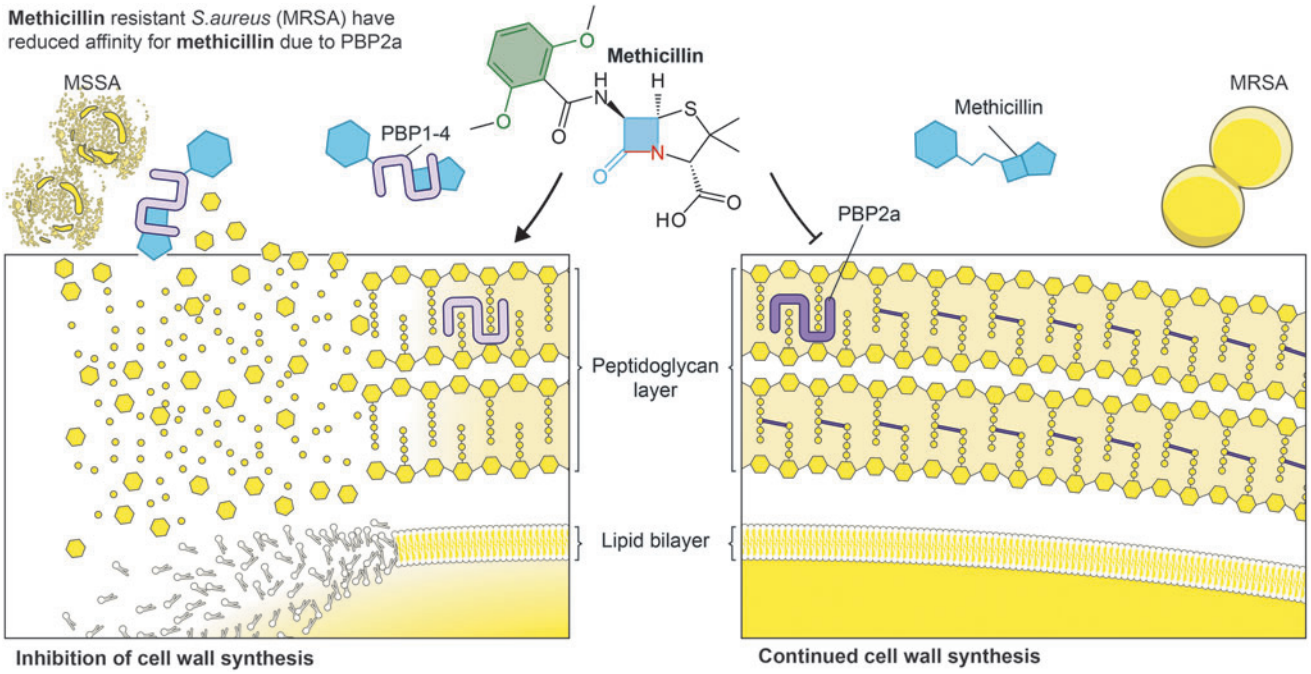

FIG. 3. ***S. aureus*** **methicillin resistance is conferred by PBP2a, which has reduced affinity for methicillin.** MSSA (*left side*) contains PBP1–4 that are readily bound by methicillin, a process that inhibits peptidoglycan and cell wall synthesis. By comparison, PBP2a has reduced affinity for methicillin, and thus PBP2a can participate in cell wall synthesis. MSSA, methicillin-susceptible *S. aureus;* PBP2a, penicillin-binding protein 2a; PBP1–4, penicillin binding proteins 1–4.

onset MRSA BSIs in the United States during the period of 2005–2012, but no change in infection rates during 2012–2017 (88). The reason for the lack of continued reduction in MRSA BSIs is not clear. There is also evidence that infection rates for MRSA are on the decline in countries outside the United States. For example, a similar decline in MRSA BSIs was reported by Wyllie *et al.* for hospitals in Oxford, United Kingdom, from 1998 to 2010 (196).

Compared with health care-associated MRSA infections, there has been little change in the rate of CA-MRSA BSIs in the United States since 2005. Many factors likely contribute to the unchanging rate of CA-MRSA infections, including the lack of a controlled environment for infection control measures and the strain of *S. aureus*. As suggested by Kourtis *et al.*, this differential is perhaps explained by a decrease in infections caused by pulsed-field type USA100 strains, which are primarily in the health care setting.

Vancomycin-intermediate S. aureus *and vancomycin-resistant* S. aureus

The glycopeptide antibiotic vancomycin continues to be important for treatment of severe MRSA infections (103). Vancomycin inhibits cell wall synthesis by binding to D-Ala-D-Ala residues of peptidoglycan precursor molecules, thereby blocking peptidoglycan synthesis (111) (Fig. 4A). As with virtually all clinically useful antibiotics, *S. aureus* can become resistant to vancomycin. Vancomycin resistance in *S. aureus* can be separated into two categories: (i) complete vancomycin resistance conferred by the *vanA* gene operon—hereafter referred to as vancomycin-resistant *S. aureus* (VRSA) and (ii) vancomycin-intermediate *S. aureus* (VISA).

In VRSA, the D-Ala-D-Ala residues of the peptidoglycan precursor molecule are replaced by D-Ala-D-Lac residues, which prevent binding of vancomycin (111) (Fig. 4B). The minimum inhibitory concentration of vancomycin for VRSA is ≥16 μg/mL, whereas that for VISA strains is 4–8 μg/mL. VRSA was first reported in the United States in 2002 (22) and in Europe in 2013 (52). To date, only 15 VRSA cases have been reported in the United States (188). VISA were first isolated in Japan in 1996 and are now widespread (67, 199). In contrast to VRSA, our understanding of the mechanisms that contribute to the development of VISA is incomplete. Previous studies have demonstrated that the VISA phenotype typically develops as a series of stepwise mutations in genes involved (directly or indirectly) in cell wall biosynthesis (78, 119) and/or occurs by transient changes in the *S. aureus* transcriptome (61, 69). These attributes, along with an often heterogeneous phenotype during the early stages of the development of VISA, make detection difficult. Although VRSA infections are difficult to treat, there appears to be a fitness cost associated with *vanA*-mediated resistance, and to date, no major outbreaks of VRSA have occurred (32, 48). By comparison, the prevalence of VISA is much greater. According to a systematic review by Zhang *et al.*, who considered 91 studies on VISA from different countries during 1997 and 2014, the prevalence of VISA has been estimated as comprising ~7.9% of MRSA isolates recovered for 2010–2014 in Asia, Oceania, India, Europe, and North America (199).

Livestock-associated MRSA

Livestock-associated MRSA (LA-MRSA) are transmitted from livestock (pigs, poultry, and cattle) to humans. This zoonotic transmission pattern was first observed in the mid-2000s and led to the introduction of the term LA-MRSA (76, 184). As is the case with *S. aureus* in general, colonization with LA-MRSA is a risk factor for subsequent infection (54). Farmers, butchers, abattoir workers, and veterinarians are especially at risk for transmission and subsequent colonization and infection (14, 72, 195). Indeed, carriage rates among pig farmers have been shown to be high in Canada, the United States, and certain European countries (54, 80, 161). In Europe and the United States, predominant LA-MRSA clones that cause human infection are classified by multilocus sequence typing (MLST or ST) as ST398 or CC398 (54, 55, 161). Genomic studies have provided strong evidence that LA-MRSA CC398 evolved from a human-tropic MSSA CC398 lineage, which lost human-specific immune modulators and acquired resistance to antibiotics (137, 181).

The need for new treatments and prophylactics

Most *S. aureus* infections can be treated successfully with appropriate antibiotics. However, MRSA often harbor resistance to multiple antibiotics, and this attribute combined with patient comorbidities makes treatment difficult and sometimes

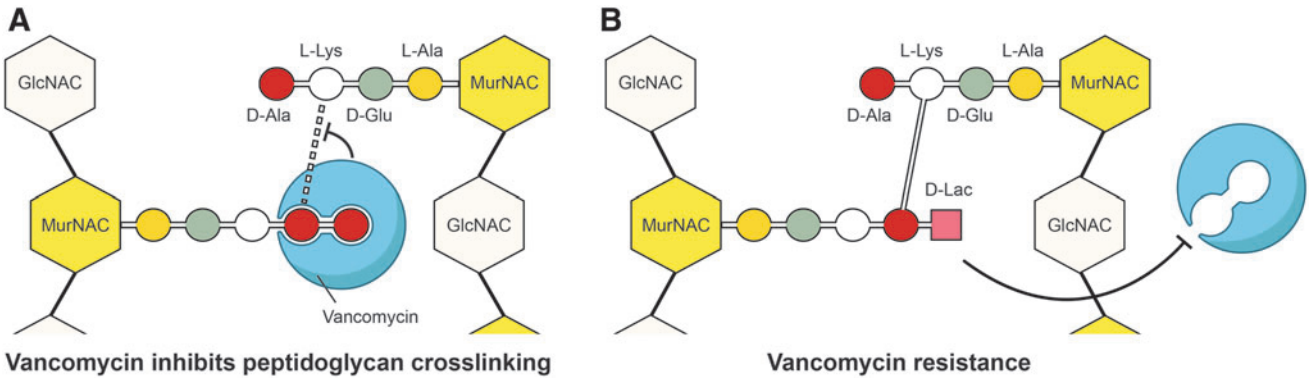

FIG. 4. Antibacterial action of vancomycin and mechanism of resistance. Vancomycin inhibits cell wall synthesis by binding to D-Ala-D-Ala residues of peptidoglycan precursor molecules **(A)**. In vancomycin-resistant *S. aureus*, D-Ala-D-Ala residues of the peptidoglycan precursor molecule are replaced by D-Ala-D-Lac residues, which do not bind vancomycin. Peptidoglycan cross-linking occurs normally **(B)**.

unsuccessful. Hence, there is a need to develop new therapeutics and prophylactics (*i.e.*, vaccines) that can reduce MRSA morbidity and mortality. The need for novel treatment options is underscored by the fact that *S. aureus* can develop resistance to the newest antimicrobials such as ceftaroline, a fifth-generation cephalosporin that is active against MRSA (190). In 2017, the WHO published a list of antibiotic-resistant priority pathogens for which new antimicrobials are needed. This list includes MRSA, VISA, and VRSA. Therapeutic and/or prophylactic approaches that target *S. aureus* virulence molecules and/or those that promote bacterial survival in the host are potentially a viable alternative to (or could be used in combination with) antibiotic therapies. Inasmuch as many *S. aureus* virulence molecules have evolved to promote evasion of human innate host defenses such as polymorphonuclear leukocytes (PMNs or neutrophils), we provide a concise overview of the role of neutrophils in host defense against *S. aureus* as relevant background.

Neutrophils in Host Defense Against Bacterial Infection

Neutrophils are short-lived, professional phagocytes, and the most abundant cells of the innate immune system. They are recruited rapidly to sites of infection or injury and are highly effective at ingesting and destroying bacteria and fungi. The importance of neutrophils in host defense is underscored by the fact that individuals with neutropenia or inherited neutrophil disorders often suffer from recurring bacterial infections and notably those caused by *S. aureus* (68).

Neutrophils comprise 55%–70% of circulating white cells in human peripheral blood. They are terminally differentiated leukocytes and have a relatively short life span during steady-state conditions. Neutrophils develop as postmitotic granulocyte precursors in bone marrow for several days (7.5 days as mitotic precursors and 6.5 days as postmitotic cells), and mature PMNs are then released into the bloodstream, where they circulate for half a day (7). These cells then enter tissues where they remain for 1–2 days, ultimately undergo apoptosis and are removed by mononuclear phagocytes.

Granulocyte turnover is extraordinary in healthy individuals, $\sim 1.8 \times 10^9$ cells/kg/day, and the majority of hematopoiesis is dedicated to their production (6). Maturation from pluripotent hematopoietic stem cells in the bone marrow is tightly regulated by transcription factors and cytokines such as PU.1, C/EBPα, granulocyte colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor. As granulocyte precursors mature in bone marrow, they develop antimicrobial capacity, which includes formation of cytoplasmic granules (7). During infection or insult, neutrophil maturation can be shortened significantly and the egress of neutrophils into the bloodstream expedited. This process is known as emergency granulopoiesis.

Stimuli that recruit neutrophils to inflammatory sites can be derived from damaged tissue, resident immune cells, and/or invading microorganisms. To reach inflamed tissue, neutrophils need to exit the vasculature. This multistep process encompasses rolling, adhesion, crawling, and transmigration and is tightly regulated by the expression of selectins and integrins present on the surface of PMNs and endothelial cells (38). After extravasation, neutrophils migrate along a chemoattractant gradient that involves polarized expression of receptors, actin polymerization, and intracellular calcium mobilization (133). Chemoattractants include bacteria-derived N-formylated peptides such as N-formyl-methionyl-leucyl-phenylalanine (fMLF), host complement components including C5a, membrane-derived lipid mediators, and chemokines (133). There is a hierarchy of neutrophil chemoattractants—for example, bacteria-derived signals such as N-formylated peptides and C5a are dominant over host-derived chemokines (133). In addition, neutrophil LYN kinase, an SRC family kinase, can function as redox sensor for H_2O_2 released by damaged host cells, thereby promoting chemotaxis (36).

Neutrophils are professional phagocytes that efficiently recognize, phagocytose, and kill invading bacteria and fungi. Pattern recognition receptors (PRRs) on the neutrophil surface recognize and bind conserved bacterial structures, such as flagellin, LPS, peptidoglycan, lipoproteins, and β-glucans. Toll-like receptors (TLRs) are among these important neutrophil PRRs. Indeed, survival of *S. aureus*-infected mice lacking TLR2 is reduced significantly compared with wild-type infected mice (173).

Although PRRs are important for recognition of microbes and priming of neutrophils for enhanced function, phagocytosis (uptake) is enhanced significantly by coating of microbes with host opsonins such as antibody and serum complement components. For example, studies performed *in vitro* have shown that phagocytosis of *S. aureus* by human neutrophils in suspension is increased significantly by opsonization in human serum (107). Such opsonins are recognized by receptors for antibody and serum complement, which when activated drive uptake or phagocytosis. Neutrophils possess several antibody Fc receptors, including three different IgG receptors and an IgA receptor, and several complement receptors (*e.g.*, CD11b/CD18 or complement receptor 3 [CR3]). During phagocytosis, microbes are sequestered within a membrane-bound organelle known as a phagosome. Phagosome maturation involves fusion with cytoplasmic granules, thereby enriching the phagosome lumen with microbicidal peptides and proteins (38). Transmembrane components of the NADPH-dependent oxidase are also enriched in the phagosome membrane by fusion with specific and gelatinase-containing granules (38). These processes are important for neutrophil microbicidal activity.

Killing of microbes within the phagosome occurs by the combination of oxygen-dependent and oxygen-independent processes (Fig. 5). Oxygen-dependent killing is dependent on an NADPH oxidase, which transfers electrons from cytoplasmic NADPH to molecular oxygen, thereby producing superoxide. The increased oxygen consumption that accompanies production of superoxide is known as the respiratory burst. Superoxide is converted rapidly to hydrogen peroxide and other microbicidal reactive oxygen species (ROS), including singlet oxygen, chloramines, hydroxyl radicals, and hypochlorous acid (38). The importance of the NADPH oxidase in host defense is underscored by the observation that patients with chronic granulomatous disease (CGD), a genetic disorder characterized by the inability of phagocytes to produce superoxide, are more susceptible to bacterial and fungal infections (68).

Oxygen-independent killing of microbes by PMNs relies on fusion of cytoplasmic granules (azurophilic and specific

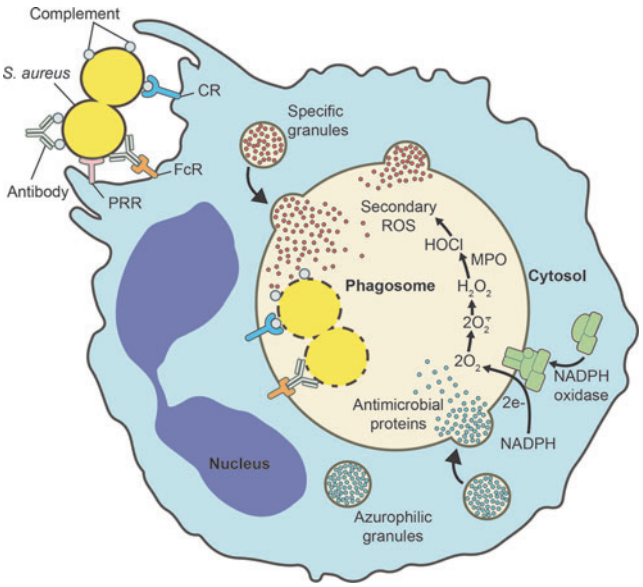

FIG. 5. Neutrophil phagocytosis and activation. Neutrophil phagocytosis of *S. aureus* and subsequent intracellular microbicidal processes. Specific and azurophilic granules fuse with the phagosome, thereby enriching the lumen of the vacuole with antimicrobial peptides and proteins. In addition, the NADPH oxidase assembles at the phagosome membrane and produces superoxide, which is converted to other ROS. CR, complement receptor; FcR, antibody Fc receptor; MPO, myeloperoxidase; PRR, pattern recognition receptor; ROS, reactive oxygen species.

granules) with the phagosome, and the resultant release of antimicrobial peptides (AMPs) and proteins into the phagosome. Azurophilic granules enrich the phagosome with numerous microbicidal molecules, including α-defensin, cathepsin, proteinase 3, elastase, and bactericidal/permeability-increasing protein, whereas specific granules contribute lysozyme, lactoferrin, calprotectin, hCAP-18 (unprocessed form of LL-37), and others (38). Additionally, some of the granule proteins restrict intraphagosomal bacterial growth. The combination of ROS and antimicrobial proteins of granules is highly effective at killing ingested bacteria and fungi.

Neutrophils also have the ability to ensnare extracellular microbes *via* the formation of extracellular traps (17). Neutrophil extracellular traps (NETs) are web-like structures consisting of chromatin, histones, and neutrophil granule proteins that are released from the cell under certain conditions or with specific stimuli (38). Decondensed chromatin acts as a ''net'' that immobilizes and kills microbes. It is worth noting that the ability of NETs to kill ensnared microbes directly has been debated (113). Although NETs can contribute to host defense, formation of NETs is accompanied by release of cytotoxic molecules that are nonspecific and can damage host tissues and/or promote development of autoimmune-mediated diseases (98).

Apoptosis and resolution of the inflammatory response

As described in brief above, neutrophils possess and/or produce numerous molecules that are cytotoxic to host cells. Therefore, multiple mechanisms exist to prevent unintended release of these molecules into host tissues. Indeed, during normal steady-state processes (noninflammatory states), neutrophils undergo constitutive (spontaneous) apoptosis at the end of their lifespan and are removed by mononuclear phagocytes (153, 154). As neutrophils undergo apoptosis, functional capacity is decreased and proinflammatory capacity is downregulated (193). Thus, apoptosis and removal of apoptotic neutrophils by macrophages is a mechanism to facilitate nonphlogistic turnover of $\sim 10^{11}$ neutrophils each day in a healthy adult.

Neutrophil apoptosis can be accelerated by phagocytosis. Watson *et al.* first reported that phagocytosis of bacteria (*Escherichia coli*) induces rapid apoptosis in human PMNs (191), and this phenomenon has since been extended to include numerous other bacteria and fungi. The acceleration of neutrophil apoptosis after phagocytosis has been termed phagocytosis-induced cell death (PICD) (198). Studies by Zhang *et al.* demonstrated that the production of neutrophil ROS is associated with PICD, and subsequent work by Kobayashi *et al.* reported that neutrophils from patients with X-linked CGD fail to undergo PICD (85, 198). These findings, coupled with earlier work by Watson *et al.* (191) and Simons *et al.* (159), in which PICD (or lack of) was dictated in part by bacteria-to-neutrophil ratios, indicate ROS are important for triggering PICD. Inasmuch as effete neutrophils need to be cleared from infected tissues, it is likely that PICD is a process that promotes safe clearance of spent neutrophils from such sites, thus contributing to the resolution of infection.

Neutrophils are the predominant cellular defense against bacteria and fungi, and therefore, it is perhaps not surprising that some bacterial pathogens have evolved means to alter normal neutrophil apoptosis and turnover. For example, pathogens such as *Anaplasma phagocytophilum* (197) and *Francisella tularensis* (155) delay neutrophil apoptosis. However, a few bacterial pathogens, including *S. aureus*, cause rapid neutrophil lysis after phagocytosis. In either case, modulation of PICD by pathogens can lead to pathogen dissemination and disease. As an example, possible outcomes of the neutrophil phagocytosis of *S. aureus* are depicted in Figure 6.

S. aureus Molecules That Target Neutrophils and Neutrophil Functions

S. aureus is known for its myriad virulence factors and immune evasion molecules, and the topic is too extensive for the purpose of this review. Therefore, we focus on selected virulence factors that target neutrophils (Table 1).

Chemotaxis inhibitors

S. aureus produces several molecules that have the potential to inhibit neutrophil chemotaxis and recruitment. For example, chemotaxis inhibitory protein of *S. aureus* blocks neutrophil chemotaxis by binding competitively to the formyl peptide receptor (FPR) and the C5a receptor (C5aR) on the neutrophil surface (134). In addition, FPRs, which bind bacteria-derived fMLF, a strong chemoattractant, are targeted by FLIPr and FLIPr-like proteins, which effectively block fMLF-mediated neutrophil chemotaxis *in vitro* (135, 136). Neutrophil recruitment is also negatively influenced by staphylococcal enterotoxin-like toxin X (SEIX) and staphylococcal superantigen-like 5, both of which bind P-selectin

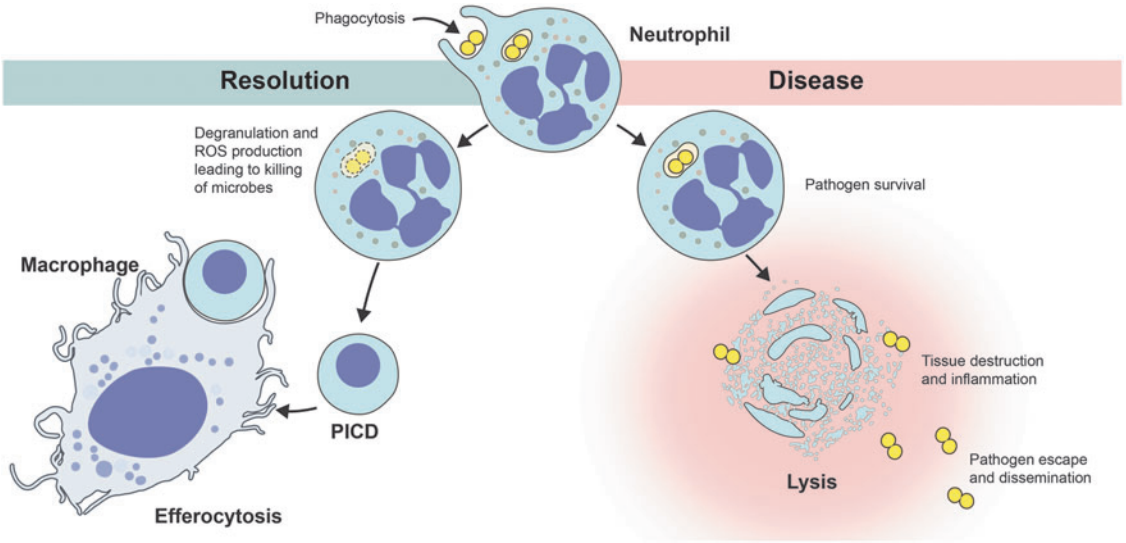

FIG. 6. Possible outcomes of neutrophil phagocytosis of *S. aureus*. Efferocytosis is the phagocytosis of cells undergoing apoptosis. PICD, phagocytosis-induced cell death.

glycoprotein ligand-1 (PSGL-1, CD162) expressed on phagocytes (12, 45). The interaction of neutrophil PSGL-1 with P-selectin on the surface of capillary endothelial cells is an important step in the initial stages of neutrophil recruitment from the bloodstream. The cysteine protease staphopain A can cleave the chemokine receptor CXCR2 (IL-8 receptor) on human neutrophils, thus preventing the binding of chemokines and subsequent chemotaxis (93).

Interaction with host opsonins

S. aureus produces numerous surface molecules that bind host serum proteins such as fibrinogen, plasminogen, and antibody. Such surface-bound host proteins can mask bacterial surface molecules, thereby reducing recognition by neutrophils. For example, *S. aureus* protein A (SpA) binds the Fc-region of IgG, an interaction that prevents antibody binding with neutrophil Fc receptors. It is because of this characteristic that SpA has been shown to decrease opsonophagocytosis (47). Indeed, *S. aureus* strains with high production of SpA were phagocytosed more slowly by neutrophils than strains that produce low SpA concentrations by comparison (132). Consistent with these observations, *S. aureus*-mutant strains lacking SpA have decreased virulence in animal infection models (40, 124, 127).

More recently, Falugi *et al.* used a mouse infection model to demonstrate that *S. aureus* SpA dampens the humoral immune response to *S. aureus* (44). Subsequent work by Pauli *et al.* (128) revealed that SpA alters the antibody response to *S. aureus* in humans, findings compatible with those of Falugi *et al.*. Inasmuch as neutralization of SpAs ability to sequester IgG and dampen antibody production would likely be beneficial to the outcome of infection and success of a potential *S. aureus* vaccine, SpA has been investigated as a potential therapeutic target. For example, Chen *et al.* showed that a recombinant monoclonal antibody (mAb) specific for SpA promotes opsonophagocytic killing of MRSA in mouse and human blood and leads to decolonization of *S. aureus* from the murine pharynx and gastrointestinal tract (25). *S. aureus* has a second immunoglobulin-binding protein, Sbi, which binds IgG and has the ability to decrease opsonophagocytosis *in vitro* (160). Compared with SpA, less is known about the role of Sbi during human infections.

S. aureus produces clumping factor A (ClfA), a fibrinogen binding protein and adhesin that promotes colonization of the host and can inhibit phagocytosis *in vitro* (65). Notably, ClfA has been targeted with numerous vaccine approaches over the past two decades (5, 18, 75, 102). Results of such studies and clinical trials have met with mixed success. Josefsson *et al.* were among the first to report that experimental vaccination with *S. aureus* ClfA or antibodies specific for ClfA moderate the severity of *S. aureus* infection in a mouse model (75). More recently, Li *et al.* found that active or passive vaccination against ClfA provided little or no protection in other mouse infection models (102). Li *et al.* hypothesized that use of Freund's adjuvant in mouse protection studies may have contributed to previous positive results with ClfA vaccine approaches. Freund's adjuvant is not used in human vaccine formulations, and results in human clinical trials have failed to provide protection and/or meet endpoint criteria. For example, DeJonge *et al.* tested the ability of intravenous immune globulin (IVIG) containing high titers of ClfA antibodies to protect infants against late-onset sepsis (37). The proportion of infants who developed late-onset sepsis was similar in placebo and IVIG treatment groups (5% and 6%, respectively) (37). Consistent with those findings, a phase II clinical trial with a humanized mAb specific for ClfA failed to reveal significant protection against *S. aureus* bacteremia (192).

Most recently, a 4-antigen *S. aureus* vaccine (SA4Ag), which includes recombinant mutant ClfA, showed promising results in preclinical studies and induced functional antibodies that persisted for up to 3 years after vaccination (9, 31). Despite these encouraging results, the vaccine was discontinued in a phase 2b clinical trial because it did not achieve predetermined endpoint objectives (PF-06290510). Inasmuch as the success of the preclinical studies for SA4Ag was in part determined by opsonophagocytic activity, the lack of efficacy in human clinical trials is perhaps not

TABLE 1. *STAPHYLOCOCCUS AUREUS* MOLECULES THAT TARGET NEUTROPHILS AND/OR NEUTROPHIL FUNCTIONS

Staphylococcus aureus molecule	*Target molecule*	*Targeted function*	*References*
Staphylococcal enterotoxin-like toxin X (SEIX)	P-selectin glycoprotein ligand-1 (PSGL-1)	Recruitment	(45)
Staphylococcal superantigen-like 5 (SSL5)	P-selectin glycoprotein ligand-1 (PSGL-1)	Recruitment	(12)
Chemotaxis inhibitory protein of *S. aureus* (CHIPS)	Formyl peptide receptor (FPR), complement component fragment 5a receptor (C5aR)	Chemotaxis	(34)
Formyl peptide receptor-like 1 inhibitor (FLIPr, FLIPr-like)	Formyl peptide receptor-like 1 (FPRL1), formyl peptide receptor (FPR)	Chemotaxis	(135, 136)
Staphopain A (ScpA)	C-X-C motif chemokine receptor 2 (CXCR2)	Chemotaxis	(93)
Capsule polysaccharide (Cps)	—	Phagocytosis	(121, 176)
Protein A (SpA)	IgG	Phagocytosis	(47, 84)
Second immunoglobulin-binding protein (Sbi)	IgG	Phagocytosis	(160)
Clumping factor A (ClfA)	—	Phagocytosis	(65)
α-Type phenol soluble modulin (PSMα)	Formyl peptide receptor 2 (FPR2), plasma membrane	PMN lysis	(130)
Panton–Valentine leukocidin (PVL)	Complement component fragment 5a receptor (C5aR1, C5aR2)	PMN lysis	(162)
Leukocidin ED (LukED)	C-X-C motif chemokine receptor (CXCR1, CXCR2), CCR5	PMN lysis	(3, 140)
Leukocidin GH/AB (LukGH/LukAB)	CD11b	PMN lysis	(41, 182)
γ-Hemolysin AB, BC (HlgAB, HlgBC)	C-X-C motif chemokine receptor (CXCR1, CXCR2, CXCR4), C-C motif chemokine receptor 2 (CCR2)	PMN lysis	(164)
Staphyloxanthin	Superoxide	ROS-mediated killing	(28, 105)
Superoxide dismutase (sodA, sodM)	Superoxide	ROS-mediated killing	(63)
Catalase (KatA)	Hydrogen peroxide	ROS-mediated killing	(110)
Alkyl hydroperoxide reductase (AhpCF)	Hydrogen peroxide	ROS-mediated killing	(30)
Staphylococcal peroxidase inhibitor (SPIN)	Myeloperoxidase (MPO)	ROS-mediated killing	(35)
Flavohaemoglobin (Hmp)	Nitrogen radicals	Nitrosative stress	(56, 141)
D-alanylation operon (Dlt)	—	Killing *via* AMPs	(131)
Peptidoglycan O-acetyltransferase (OatA)	—	Killing *via* AMPs	(10)
Multiple peptide resistance factor (MprF)	—	Killing *via* AMPs	(92, 129)
Staphylokinase	α-defensins	Killing *via* AMPs	(74)
Aureolysin	Cathelicidin LL37	Killing *via* AMPs	(158)
Extracellular adherence protein (Eap), extracellular adherence protein homologue (EapH, EapH2)	Elastase, proteinase 3, and cathepsin G	Killing *via* AMPs	(167)
ABC transporter (VraFG)	—	Killing *via* AMPs	(99)
Nuclease	NETs	Trapping by NETs	(11)
Adenosine synthase A (AdsA)	NETs	Trapping by NETs	(177)

AMPs, antimicrobial peptides; NETs, neutrophil extracellular traps; PMN, polymorphonuclear leukocyte; ROS, reactive oxygen species.

surprising. Although *S. aureus* produces many molecules that have the ability to inhibit phagocytosis *in vitro*, it has long been known that *S. aureus* is ingested readily by phagocytes—most notably neutrophils—from healthy adults (18–65 years, the population used in the clinical trial) (148, 185). Thus, a vaccine directed largely to promote opsonophagocytic activity is not optimal. More work is needed to fully understand the contribution of ClfA in human infections.

Cytolytic toxins

Virtually all *S. aureus* strains have the ability to produce multiple cytolytic toxins (157). Alpha-hemolysin (or alpha-toxin, Hla) is one of the best characterized and highly conserved *S. aureus* toxins (13). The role of Hla in virulence has been tested in multiple animal infection models over the years, including models of *S. aureus* SSTI, bacteremia, peritonitis, and pneumonia (19, 79, 172). The toxin contributes significantly to virulence in each of these models and is therefore a reasonable target for therapeutic approaches. Indeed, passive and active vaccination approaches that target Hla have shown good success in animal infection models especially when combined with other antigens (1, 39, 66, 70, 79, 114). Early studies by Adlam *et al.* demonstrated that immunization of rabbits with purified Hla protects rabbits against lethal experimental mastitis (1). Subsequent work by Menzies and Kernodle demonstrated that rabbit antiserum

elicited by immunization with an Hla toxoid protects mice against lethal challenge with *S. aureus* (114). Consistent with those findings, Bubeck Wardenburg and Schneewind showed that immunization of mice with Hla toxoid protects animals against *S. aureus* pneumonia (20). Kennedy *et al.* then reported that active and passive vaccine approaches directed against Hla protect mice from severe and soft tissue infections caused by the USA300 epidemic strain (79). Collectively, these studies set the stage for development of therapeutic approaches directed against Hla.

Indeed, the human anti-Hla mAb MEDI4893 has been tested in various animal infection models both as a prophylactic and a therapeutic treatment option and been found to reduce disease severity significantly, especially when combined with antibiotics (66). A phase 1 study in humans revealed that intravenous administration of MEDI4893 resulted in long-lasting anti-Hla neutralizing antibody levels (152), and a phase 2 study demonstrated reduction of *S. aureus* pneumonia in high-risk intensive care unit patients treated with MEDI4893 (51). Thus, Hla is a promising target for passive and active immunization approaches.

The bicomponent leukocidins are group of well-studied *S. aureus* cytotoxins that cause lysis of white blood cells. They consist of two subunits, known as S and F, which are secreted separately but assemble in the target cell membrane to form β-barrel pores that can disrupt cell homeostasis and cause cell lysis (157). *S. aureus* bicomponent leukocidins include Panton–Valentine leukocidin (PVL), leukocidin ED (LukED), LukGH/LukAB, gamma-hemolysin AB (HlgAB), and HlgCB (163). Each has a slightly different host cell target spectrum, and some leukotoxins are host specific and leukocyte lysis is species specific (163). A number of leukocyte transmembrane receptors for these leukotoxins have been identified recently, including C5a and IL-8 receptors, and CD11b (163). The contribution of leukocidins to *S. aureus* virulence has been investigated extensively in animal infection models. Based on such studies, one can argue that each of these leukotoxins has a role in *S. aureus* virulence under certain conditions. That said, the role played by each of these molecules in human infections remains unknown or incompletely determined and more work is needed.

Despite this lack of knowledge, *S. aureus* cytotoxins are being considered as targets for possible therapeutic approaches. Studies by Rouha *et al.* showed that a combination of two human mAbs (named ASN100), which are together specific for six *S. aureus* cytotoxins (Hla, LukSF-PV, LukED, HlgAB, HlgCB, and LukGH/AB), inhibits lysis of host epithelial cells and leukocytes—including PMNs—*in vitro* (150). These mAbs prevented lethality in a rabbit model of severe *S. aureus* pneumonia (169), and more recently, showed promising results for safety, tissue penetration, and functional activity in healthy human volunteers (109). The approach to target *S. aureus* cytotoxins was corroborated by Vu *et al.*, who demonstrated the ability of MEDI4893 (the aforementioned anti-Hla antibody) and a mAb specific for PVL, LukED, and HlgAB/CB to moderate the severity of disease in a rabbit model of *S. aureus* necrotizing pneumonia (187). Tran *et al.* showed further that a combination of cytotoxin antigens (Hla and PVL subunit toxoids) led to greater protection against lethal USA300 pneumonia in rabbits than any of the antigens alone, findings compatible with earlier work by Rouha *et al.* and Stulik *et al.* (150, 169, 179). Taken together, these studies provide compelling support for a prophylactic and/or therapeutic that targets multiple *S. aureus* cytotoxins by active or passive vaccination.

α-Type phenol-soluble modulin (PSMα) peptides are secreted, short amphipathic α-helical peptides that can lyse many types of host cells, including neutrophils (189). In contrast to the bicomponent leukotoxins, PSMα-mediated cytolysis is likely receptor independent and attributed to its surfactant-like properties. However, at sublytic concentrations, PSMα peptides can bind FPR2 on neutrophils and elicit proinflammatory processes (26, 90). Although *psm* loci are present in the core genome of virtually all *S. aureus* lineages, high PSM expression levels are associated with the most prominent CA-MRSA strains, suggesting a link between PSM production and the success of CA-MRSA (100).

The expression of PSMα peptides as well as other important *S. aureus* virulence factors is regulated by the accessory gene regulator (Agr) quorum-sensing system, which has also been investigated as a potential therapeutic target (194). For example, Sully *et al.* identified a small-molecule inhibitor, savarin, that targets AgrA, the transcription regulator of the *agr* operon, and thus prevents transcription of *hla*, *psm-α*, and *lukS/F-PV* (170). In addition to blocking lysis of human PMNs *in vitro*, administration of savarin to mice increased bacterial clearance and reduced tissue injury in *S. aureus* skin infection models (170). Inasmuch as Agr regulates the expression of a number of *S. aureus* virulence factors, targeting the Agr quorum sensing system is potentially a promising approach for the development of novel prophylactics and therapeutics directed to moderate severity of *S. aureus* infections.

Moderation of ROS and resistance to AMPs

S. aureus possesses multiple factors to protect itself from ROS and reactive nitrogen radicals, including genes encoding superoxide dismutases (SodA and SodM), catalase (KatA), alkyl hydroperoxide reductase (AhpCF), staphylococcal peroxidase inhibitor (SPIN), flavohaemoglobin (Hmp), and staphyloxanthin. Some of these molecules are upregulated following phagocytosis of *S. aureus* by human neutrophils (185), and the importance of some of these proteins has been tested in animal infection models. For example, SPIN is highly conserved among *S. aureus* lineages, and expression is upregulated within 20 min after phagocytosis (35). SPIN binds MPO and actively prevents formation of hypochlorous acid, the product of the MPO-halide system originally described by Seymour Klebanoff (35). A SPIN deletion mutant is more susceptible to killing by neutrophils compared with the *S. aureus* wild-type strain (35).

The structural similarity between staphylococcal dehydrosqualene synthase (CrtM), which is important for production of staphyloxanthin, and human squalene synthase (SQS), an enzyme involved in cholesterol synthesis, has been exploited as a potential therapeutic by Liu *et al.* (104). Certain phosphosulfonates, which act as cholesterol synthesis inhibitors in humans, inhibit staphyloxanthin synthesis in *S. aureus* and thereby render the bacterium more susceptible to killing by ROS (104). Mice treated with phosphosulfonate had reduced bacterial burden in a mouse kidney infection model. Considering that cholesterol-lowering drugs are widely

used in humans, exploiting antistaphylococcal capacity is an intriguing therapeutic approach that merits further research.

In addition to the ability to moderate ROS, *S. aureus* has evolved multiple mechanisms to circumvent killing by AMPs and proteins. Here, we provide selected examples of these *S. aureus* AMP resistance mechanisms. For instance, *S. aureus* can modify cell wall composition, cleave AMPs, and bind and/or export them from the cytoplasm *via* efflux pumps. *S. aureus* encodes a three-component AMP sensing system that controls expression of anti-AMP molecules (99, 101). Cell wall modifications include D-alanylation of teichoic acid and incorporation of cationic lysyl-phosphatidyl glycerol into the bacterial membrane as a means to neutralize the negative surface charge and thus help repel cationic AMPs such as neutrophil defensins (131). These processes are mediated by the *S. aureus* DltABCD system and multiple peptide resistance factor (129, 131).

O-acetylation of muramic acid by staphylococcal OatA confers resistance to lysozyme (10), and staphylokinase, aureolysin, and V8 protease inactivate AMPs by direct binding and/or proteolysis (74, 158). More recently, Stapels *et al.* discovered that *S. aureus* extracellular adherence protein (Eap) and homologs EapH1 and EapH2 inhibit the neutrophil serine proteases elastase, proteinase 3, and cathepsin G (167). These Eap proteins inhibit neutrophil serine protease activity *in vitro* and contribute to virulence in a mouse infection model (167). *S. aureus* has also evolved the means to escape NETs. For example, Berends *et al.* discovered that *S. aureus* nuclease (encoded by *nuc*) facilitates escape from NETs (11). Subsequent work by Thammavongsa *et al.* reported that nuclease coupled with *S. aureus* adenosine synthase A converts NETs into deoxyadenosine, which can initiate cell death in immune cells (177).

Although the majority of *S. aureus* ingested by neutrophils are killed, some of the ingested microbes can survive long enough to ultimately cause neutrophil lysis. This phenomenon varied depending on the *S. aureus* strain but is perhaps not unexpected considering the many mechanisms used by *S. aureus* to moderate the effects of neutrophil microbicides.

Neutrophil Lysis After Phagocytosis of *S. aureus*

As described above, many bacterial pathogens have the ability to alter the fate of neutrophils, and *S. aureus* is one such pathogen. In the early 1950s, Rogers and Tompsett reported that not all phagocytosed *S. aureus* are killed by neutrophils *in vitro* (148). Moreover, there was significant destruction/lysis of human neutrophils 3–4 h after phagocytosis of *S. aureus* (148). Subsequent work by Rogers and Melly showed that although 99% of *S. aureus* were ingested by human granulocytes *in vitro*, there was $\sim$50% bacterial survival (112, 147). Rogers also demonstrated that staphylococci contained within leukocytes cause persistent bacteremia in a rabbit model (146). The overarching idea was that any ingested staphylococci that survived after phagocytosis could serve as a source for persistent infection.

Almost 50 years later, Gresham *et al.* demonstrated that *S. aureus*-containing neutrophils can establish *S. aureus* infection in mice, suggesting release of the pathogen from neutrophils (60). Voyich *et al.* found that prominent CA-MRSA strains, especially the USA300 and USA400 strains, cause rapid lysis of human neutrophils following phagocytosis (185, 186). The enhanced ability of these CA-MRSA strains to evade killing by neutrophils suggests that they have enhanced virulence capacity, an attribute perhaps linked to their ability to cause infections in otherwise healthy individuals. Collectively, these previous observations have provided strong support to the idea that lysis of neutrophils after phagocytosis of *S. aureus* contributes to the pathogenesis of infection.

Given the potential importance of the *S. aureus*–neutrophil lysis phenomenon, there has been a recent effort to elucidate the mechanism for this process. The mechanism (or mechanisms) underlying neutrophil lysis after phagocytosis involves molecules produced by *S. aureus* within the phagosome, host cell processes triggered by *S. aureus*, or a combination of both. Kobayashi *et al.* showed that neutrophil lysis after phagocytosis of USA300 occurs independent of caspases and ROS produced by NADPH oxidase, thus eliminating the possibility of an apoptosis-like form of cell death (83). Furthermore, neutrophil lysis increased over time, was dependent largely on bacteria-to-neutrophil ratios, and required viable *S. aureus* (83). Interestingly, the authors found the phagosome membrane remained intact until the point of lysis (83). In subsequent work, Greenlee-Wacker *et al.* provided evidence that neutrophil lysis after *S. aureus* phagocytosis occurs by necroptosis or programmed necrosis (59). Further characterization of the neutrophil lysis phenomenon by the same authors indicated that lysis occurs independent of classical necroptosis molecules but requires RIPK-3 (58). Therefore, the role of specific neutrophil signal transduction pathways remains incompletely determined.

At least three *S. aureus* secreted toxins have been shown to contribute to lysis of neutrophils after phagocytosis. First, Ventura *et al.* found that an isogenic USA300 *lukGH* deletion strain caused significantly less neutrophil lysis after phagocytosis compared with that by the wild-type strain (182). The contribution of LukGH/LukAB to this process was verified and extended by DuMont *et al.* (42). Pang *et al.* compared USA300 wild-type and isogenic *hla* deletion strains to show that Hla contributes to PMN lysis after phagocytosis of *S. aureus* (125). It is noteworthy that Hla and LukGH/LukAB require interaction with specific surface receptors to form cytolytic membrane pores. Although these receptors would be present and in the correct orientation in the phagosome membrane, they are inaccessible from the cytoplasmic face of the plasma membrane and thus should be unable to cause cytolysis directly. However, these findings are compatible with the notion that interaction of Hla and/or LukGH/LukAB with host receptors lining the inside of the phagosome membrane triggers host cell death through an as yet undefined signaling pathway. Indeed, Hla has been shown recently to activate the inflammasome in macrophages, thus providing support to the idea that these cytolytic toxins can activate processes mediated by host signal transduction (29).

Finally, Surewaard *et al.* reported that PSMα peptides, which lack the requirement for membrane receptor interaction to effect cytolysis, contribute to neutrophil lysis after phagocytosis of *S. aureus* strain MW2, the prototype CA-MRSA strain (171). Whether the PSMα peptides target membranes directly or trigger signals that lead to neutrophil death remains incompletely determined. Clearly, more work is needed to fully understand the mechanism(s) that underlie rapid neutrophil lysis after phagocytosis.

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Address correspondence to:
Dr. Frank R. DeLeo
Laboratory of Bacteriology
Rocky Mountain Laboratories
National Institute of Allergy and Infectious Diseases
National Institutes of Health
903 South 4th Street
Hamilton, MT 59840
USA

E-mail: fdeleo@niaid.nih.gov

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Abbreviations Used

AMPs = antimicrobial peptides
BSI = bloodstream infection
CA-MRSA = community-associated methicillin-resistant *Staphylococcus aureus*
CC = clonal complex
CGD = chronic granulomatous disease
CHIPS = chemotaxis inhibitory protein of *S. aureus*
ClfA = clumping factor A
Eap = extracellular adherence protein
fMLF = N-formyl-methionyl-leucyl-phenylalanine
FPR = formyl peptide receptor
FPR2 = formyl peptide receptor 2
G-CSF = granulocyte colony-stimulating factor
GM-CSF = granulocyte–macrophage colony-stimulating factor
Hla = α-hemolysin or alpha-toxin
IVIG = intravenous immune globulin
LA-MRSA = livestock-associated MRSA
Luk = leukocidin
LukED = leukocidin ED
mAb = monoclonal antibody
MLST or ST = multilocus sequence typing
MprF = multiple peptide resistance factor
MRSA = methicillin-resistant *S. aureus*
NADPH = nicotinamide adenine dinucleotide phosphate
NETs = neutrophil extracellular traps
PBP2a = penicillin-binding protein 2a
PICD = phagocytosis-induced cell death
PMN = polymorphonuclear leukocyte
PRR = pattern recognition receptor
PSGL-1 = P-selectin glycoprotein ligand-1
PSMα = α-type phenol soluble modulin
PVL = Panton–Valentine leukocidin
ROS = reactive oxygen species
Sbi = second immunoglobulin-binding protein
ScpA = Staphopain A
SCV = small colony variant
SEIX = staphylococcal enterotoxin-like toxin X
SpA = *S. aureus* protein A
SPIN = staphylococcal peroxidase inhibitor
SSL5 = superantigen-like 5
SSTI = skin and soft tissue infections
TLR = toll-like receptor
VISA = vancomycin-intermediate *S. aureus*
VRSA = vancomycin-resistant *S. aureus*