Development of Antistaphylococcal Vaccines

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Staphylococcus aureus is frequently isolated from both hospital-acquired and community-acquired infections, and the emergence of antibiotic resistance among clinical isolates has made treatment of staphylococcal infections difficult. This scenario has sparked renewed interest in the development of a vaccine for individuals at high risk for staphylococcal infections. As part of the effort to develop a multicomponent vaccine against *S. aureus*, several vaccine candidates are currently being evaluated in animal models of staphylococcal infection or in human clinical trials. The most promising candidates to date include adhesins (fibronectin-binding protein, collagen-binding protein, and fibrinogen-binding protein [clumping factor]), a nontoxic alpha toxin mutant, and capsular polysaccharides type 5 and 8.

Introduction

Staphylococcus aureus is an important bacterial pathogen that is responsible for a diverse spectrum of human and animal infections. Superficial infections caused by this microbe include impetigo, cellulitis, and folliculitis, whereas more invasive staphylococcal infections include osteomyelitis, septic arthritis, pneumonia, bacteremia, and endocarditis. Placement of a prosthetic device enhances the risk for staphylococcal infection. *S. aureus* infections are often nosocomial, but reports of community-acquired infections have increased. Alternative strategies for the prevention and treatment of staphylococcal infections are needed because of the emergence of antibiotic-resistant strains, including the recent identification of strains with reduced susceptibility to glycopeptide antibiotics.

This scenario has sparked renewed interest in the development of an *S. aureus* vaccine for individuals at risk for staphylococcal infections. Table 1 lists populations that might be suitable candidates for immunization against *S. aureus* infection. The list includes patients with a variety of predisposing factors and groups at risk for trauma. Nasal

carriage of *S. aureus* is a risk factor for acquisition of staphylococcal infection; thus, a vaccine that would prevent nasal carriage of *S. aureus* would benefit hospital personnel as well as the patient populations listed in Table 1.

Progress in the development of a vaccine to protect against *S. aureus* has been limited by our inadequate understanding of what constitutes a protective host immune response to this microbe. In fact, evidence that recovery from *S. aureus* infections confers immunity to subsequent episodes of staphylococcal diseases is lacking. Nonetheless, because of the limited number of therapeutic agents effective against the *Staphylococcus*, efforts are ongoing to design an *S. aureus* vaccine by targeting known virulence factors of this organism. These factors are listed in Table 2 and include surface proteins, polysaccharides, exoproteins, and toxins elaborated by this pathogen.

Protein Vaccines

Staphylococcus aureus produces a variety of cell wall-associated proteins that interact with extracellular matrix proteins of the host. These staphylococcal adhesins have been dubbed "microbial cell surface components recognizing adhesive matrix molecules" (MSCRAMMS). Staphylococcal proteins that bind to fibronectin, fibrinogen, and collagen (Fig. 1) have been investigated as components of vaccines to protect against experimental *S. aureus* infections in laboratory animals.

Fibronectin-binding proteins

Most *S. aureus* strains carry two fibronectin-binding proteins (FnBps) arranged in tandem on the bacterial chromosome. The binding motifs within FnBpA (and also FnBpB) consist of three repeat regions (37-38 amino acids long) designated D1-3 (Fig. 1). The D-repeats of FnBpA and FnBpB are highly homologous. Schennings *et al.* [1] prepared a fusion protein by cloning the *lacZ* gene encoding β -galactosidase (gal) in frame with the D1-3 repeat region of the *S. aureus fnbA*. Rats were immunized with the gal-FnBp in Freund's complete adjuvant, and the resulting antibodies blocked the binding of *S. aureus* to immobilized fibronectin in vitro. Immunization with the fusion protein provided modest protection against experimental endocarditis in rats compared with nonimmunized animals. Likewise, mice immunized with gal-FnBp (with Freund's adjuvant) showed fewer cases of severe mastitis than

Table 1. Potential candidates for active or passive immunization against *Staphylococcus aureus* infections

Surgical patients
Intravenous drug users
Peritoneal dialysis patients
Premature neonates
Immunocompromised individuals
Hospital personnel
Diabetics
Patients with prosthetic implants
Hemodialysis patients
Military personnel
Firefighters
Law enforcement agents

did control mice (immunized with adjuvant alone), and fewer bacteria were recovered from the mammary glands of vaccinees than from those of control mice [2].

In a more recent study by Brennan *et al.* [3], a truncated form of the D2 peptide (amino acids 1–30) derived from *S. aureus* FnBpB was expressed on the surface of the icosahedral cowpea mosaic virus (CPMV). Mice and rats were immunized subcutaneously with the recombinant plant virus-based recombinant vaccine (CPMV-D) with adjuvant. All of the mice produced high titers of FnBp D2-specific IgG2a and IgG2b, and little IgG1. Antibodies to CPMV-D inhibited the binding of *S. aureus* to purified fibronectin, but the opsonic activity of these antibodies against *S. aureus* was not assessed. To determine whether antibodies to the recombinant CPMV-D particles provided protection against *S. aureus* infection, Rennermalm *et al.* [4] immunized rats subcutaneously with either 250 µg to 500 µg of CPMV-D or 150 µg to 500 µg of CPMV alone. The animals were immunized on days 0, 14, and 28, and the antigens were injected with the adjuvant QS-21. Protection against staphylococcal infection was evaluated in a coupled arthritis/endocarditis model. A corticosteroid was injected into the right temporomandibular joint, and *S. aureus* was injected intravenously. As a result, the rats developed septic arthritis in the joint and a continuous low-grade bacteremia. One week later, the rats were catheterized to induce the development of vegetations on the aortic valve. The animals were killed 24 hours later, and the vegetations, mandibular joints, and kidneys were homogenized and cultured quantitatively. The number of bacteria recovered from infected joints (~ 6.5 log colony-forming units [CFU] *S. aureus*) was similar for the two groups, but the median CFU/valve (3.2 logs) for animals immunized with CPMV-D was approximately 1000-fold lower than that for control animals given virus alone. Kidney cultures from both groups yielded high numbers of organisms, and the number of *S. aureus* recovered from the kidneys of immunized rats (7.2 log CFU) was only 1 log lower than the number in control rats (8.2 log CFU).

In a separate study [5], rats and mice were immunized intranasally with purified recombinant CPMV-D. The non-

replicating recombinant viral particles induced high titers of FnBp-specific IgG in the rodent sera. Peptide-specific IgA and IgG could also be detected in the bronchial, intestinal, and vaginal lavage fluids. The collective results of this group of studies demonstrate the potential of FnBp and plant virus-based vaccines to elicit partial protection against *S. aureus* infections.

Collagen-binding protein

The staphylococcal collagen-binding protein (Cna) is present on 30% to 60% of *S. aureus* strains and represents an important virulence factor in the pathogenesis of staphylococcal septic arthritis, endocarditis, and keratitis [6–8]. Immunization with purified Cna did not protect rats against staphylococcal endocarditis [1]. However, Nilsson *et al.* [9••] demonstrated that vaccination with the domain A fragment of the *S. aureus* collagen adhesin (Cna-A) protected mice against sepsis-induced death. Mice were injected subcutaneously with 100 µg of protein (in Freund's complete adjuvant) and with two booster doses (without adjuvant). Mice immunized with the recombinant protein or bovine serum albumin (BSA) were challenged intravenously with 2×10^7 CFU of the Cna-positive *S. aureus* strain Phillips. After 14 days, mortality in the group vaccinated with Cna-A was only 13%, compared with 87% in the control group immunized with BSA. Among mice given a sublethal inoculum (6×10^6 CFU), the control animals exhibited a significant decrease in their body weight compared with the Cna-A-immunized mice, but there was no significant decrease in the development of septic arthritis between the two groups of animals. The investigators demonstrated that the protective effect against *S. aureus*-induced lethality was antibody mediated by passive immunization of naïve mice with rat antibodies to the collagen adhesin. Compared with mice given antibodies to BSA, mice passively immunized with Cna-A antibodies were protected against sepsis-induced death.

Fibrinogen-binding proteins

Staphylococcus aureus produces a number of cell-associated proteins that bind to fibrinogen. Two of the best-characterized proteins are clumping factor A and B (ClfA and ClfB). ClfA has been shown to be an important virulence determinant in a rat model of staphylococcal endocarditis [10]. In unpublished studies, Bayer and coworkers evaluated whether antibodies to ClfA would protect rabbits in a catheter-induced model of *S. aureus* endocarditis. Rabbits were immunized four times with 50 µg to 100 µg of a recombinant peptide encompassing the ligand-binding domain A of ClfA adsorbed to alum or alum alone. The rabbits were catheterized and challenged intravenously 24 hours later with 8×10^4 CFU of a methicillin-resistant *S. aureus* isolate (strain 67-0). The ClfA-immunized rabbits had significantly fewer staphylococci in their blood, vegetations, and kidneys compared with control animals. In a related study, scientists at Inhibitex, Inc. (Alpharetta, GA) evalu-

Table 2. Potential virulence determinants of *Staphylococcus aureus* that could be targeted for vaccine production

Cell-associated components	Exoenzymes	Exotoxins
Protein A	Coagulase	Leukocidin
Capsular polysaccharide	Hyaluronate lyase	Enterotoxins (A to E, G to P)
Peptidoglycan	Lipase	Exfoliative Toxin
Poly-N-acetyl glucosamine or polysaccharide	Nuclease	Toxic shock syndrome toxin-I
intercellular adhesin	Proteases	Alpha toxin
Adhesins for		Beta toxin
Fibronectin		Gamma toxin
Fibrinogen		Delta toxin
Collagen		
Elastin		
Vitronectin		
Bone sialoprotein		
Thrombospondin		
Plasmin		
von Willebrand factor

Figure 1. Structural organization of the MSCRAMM proteins (a) FnBPA; (b) Cna; and (c) ClfA of *Staphylococcus aureus*. *S* represents the signal sequence; *R* represents Ser-Asp dipeptide repeats; *W* represents the wall-spanning region; and *M* represents the membrane-spanning region and positively charged residues. The positions of the LPXTG motif and the A, B, C, and D domains are indicated. Asterisks indicate ligand-binding domains. (Adapted from Foster and Hook [35].)

ated the ability of a ClfA-specific monoclonal antibody (mAb) to protect mice against lethality in an intravenous challenge model of infection. The animals were passively immunized with 0.3 mg of purified mAb 12-9 (IgG1) 18 hours prior to challenge with 2×10^9 CFU of *S. aureus* 67-0. Seven days after bacterial challenge, 70% of the mice treated with 12-9 mAb survived, as compared with only 30% of the animals given an isotype control mAb (J Patti, Personal communication).

Toxoids

Alpha toxoid

Alpha toxin, a pore-forming and hemolytic exoprotein produced by most strains of *S. aureus*, is a major staphylococcal virulence determinant. Human cells that demonstrate a high susceptibility to the lethal effects of this toxin include platelets, endothelial cells, and monocytes. Antibodies raised to alpha toxin detoxified with formaldehyde have been shown to protect monkeys against the toxic and lethal action of purified alpha toxin [11]. However, animals immunized with alpha toxoid and challenged with live bacteria are not generally protected from infection with

alpha toxin-producing strains of *S. aureus*, although the clinical severity of their disease may be less than that in nonimmunized controls [2,12–14]. Menzies and Kernodle [15] constructed a nontoxic alpha toxin mutant by replacing a histidine with a leucine at amino acid 35 (H35L toxin). The investigators demonstrated that passive immunization of mice with rabbit antiserum to the nontoxic alpha toxin mutant protected the animals against lethality induced by intraperitoneal injection of purified alpha toxin. Mice given preimmune serum died between 2 and 4 hours after challenge, whereas animals given immune serum were alive at 24 hours (when the experiment was terminated). Antibodies to the H35L toxin also prevented the lethality at 24 hours induced by an alpha toxin-overproducing strain of *S. aureus*. However, two of the six surviving mice developed intra-abdominal abscesses 10 days after challenge.

Enterotoxins (superantigens)

Staphylococcus aureus produces a wide variety of superantigen exoproteins, including toxic shock syndrome toxin-1, exfoliative toxins A and B, and enterotoxins A to P. The native proteins would not be appropriate vaccine compo-

nents since superantigens act as potent oligoclonal T-cell activators, resulting in the massive release of cytokines. Administration of the native molecules would likely disturb the homeostasis of the immune system and possibly result in tissue damage, septic shock, or death. However, mutant forms of the enterotoxin proteins devoid of their superantigenic properties may still express antigenic determinants that could be the target of neutralizing antibodies. Nilsson *et al.* [16•] generated a genetically modified staphylococcal enterotoxin A (SEA) mutated in a hydrophobic loop dominating the interface with the major histocompatibility complex class II locus. Mice were injected subcutaneously with one to three doses of recombinant SEA (10 µg) emulsified in Freund's adjuvant. The mice were inoculated intravenously with $5-8 \times 10^7$ CFU of an SEA-producing *S. aureus* 1 to 5 weeks after the last immunization. In three separate experiments, mice injected with the recombinant protein showed a significantly higher survival rate than mice immunized with BSA. Passive administration of SEA-specific rat antibodies to naïve mice also protected the animals against lethality due to staphylococcal sepsis. After 5 days, however, the differences in survival between the two groups of mice diminished, suggesting that additional doses of antibody might be necessary to extend the protection afforded by passive immunization.

Similarly, Lowell *et al.* [17] demonstrated that immunization with a formalinized staphylococcal enterotoxin B (SEB) toxoid formulated with meningococcal outer membrane protein proteosomes was immunogenic in mice. Proteosome-toxoid delivered intranasally or intramuscularly afforded protection against lethal challenge with purified SEB delivered by the parenteral or respiratory route to D-galactosamine-sensitized mice. In a subsequent study, Lowell *et al.* [18] immunized nonhuman primates intramuscularly with the same proteosome-toxoid vaccine. Booster doses were administered by either the intramuscular or intratracheal route. One month after the last immunization, the primates were challenged by the aerosol route with 15 times the 50% lethal dose of purified SEB. Aerosol exposure to SEB results in gastrointestinal symptoms, lethargy, shock, and death in naïve animals. However, the proteosome-SEB toxoid vaccine protected 100% of monkeys against severe symptomatology and death due to aerosolized SEB intoxication. Furthermore, protection against lethality was achieved whether the booster immunizations were administered by the intramuscular or by the intratracheal route.

A recent report by Stiles *et al.* [19] described the use of a recombinantly attenuated SEB vaccine (SEBv) to immunize mice by the mucosal route. Animals were given three vaccinations of SEBv (20 or 2.5 µg) administered nasally or orally with or without immunoadjuvant cholera toxin. The mice were challenged 3 weeks later with a lethal aerosol or intraperitoneal dose of SEB together with 75 µg of lipopolysaccharide given intraperitoneally. Their results indicated that nasally

administered SEBv (20 µg) plus 5 µg cholera toxin was 100% protective against an intraperitoneal or aerosol toxin challenge. Immunization by the nasal route resulted in better seroconversion rates and protection than did immunization by the oral route.

Staphylococcal enterotoxins that are devoid of their superantigenic properties may be useful vaccine candidates for protecting against superantigens used as potential biological warfare agents. However, the many different superantigens produced by this organism and the variability that exists between strains limit the utility of these toxins as constituents of a multicomponent staphylococcal vaccine.

RNAIII-activating Protein

The expression of many virulence factors in *S. aureus* is controlled by the global regulator *agr*. RNAIII, the effector molecule of the *agr* response, upregulates transcription of many staphylococcal exoproteins and capsule and represses transcription of certain surface-protein genes. In addition to the octapeptide autoinducing molecules described by Ji *et al.* [20], Balaban and Novick [21] described a 38-kD RNAIII-activating protein (RAP) that is secreted into the culture medium. In 1998, Balaban *et al.* [22•] reported that RAP might serve as a vaccine target to prevent *S. aureus* infections. They purified RAP from postexponential staphylococcal culture supernatants and used this as an immunogen in mice. Animals infected with three 10-µg doses of the protein together with adjuvant were compared with control animals (given adjuvant alone) in a mouse model of *S. aureus* cellulitis induced by the heavily encapsulated strain Smith diffuse. Only 30% of mice injected with RAP and challenged with *S. aureus* had skin lesions, compared with 72% of the control animals. Lesions that developed in the RAP-vaccinated mice were smaller than those that developed in the control animals. Likewise, fewer immune mice (3%) than control mice (22%) died as a result of the experimental infection. The antibody concentrations correlated inversely with lesion size, suggesting that antibodies conferred protection. The novel feature of Balaban *et al.*'s approach is that immunization with RAP resulted in a decrease in the incidence and severity of staphylococcal infections by interfering with bacterial virulence rather than by targeting bacterial growth. Whether immunization with RAP will protect against systemic infections caused by *S. aureus* and whether it will be effective against clinical isolates of *S. aureus* remain to be determined, since no follow-up studies on this molecule have been reported.

Capsular Polysaccharide

Several published reports have indicated that antibodies elicited to the capsular polysaccharides (CPs) of *S. aureus* are protective in animal models of experimental staphylococcal infection. Because polysaccharides are poorly

Figure 2. Structural composition of serotype 5 (CP5) and serotype 8 (CP8) capsular polysaccharides.

immunogenic and generally elicit a T-cell-independent antibody response, Fattom *et al.* [23] conjugated the most prevalent *S. aureus* CP types (serotypes 5 and 8) (Fig. 2) to recombinant *Pseudomonas aeruginosa* exotoxin A. The conjugate vaccines were highly immunogenic in mice and humans and induced antibodies that opsonized encapsulated *S. aureus* for phagocytosis [23,24]. Antibodies elicited by immunization with the CP5 and CP8 conjugate vaccines were protective in a mouse model of *S. aureus* lethality and disseminated infection [25]. Similarly, intraperitoneal administration of antibodies to the *S. aureus* conjugate vaccine protected rats against infection in a catheter-induced model of staphylococcal endocarditis if the animals were challenged by the intraperitoneal route [26].

Active immunization of humans with the CP5/CP8 conjugate vaccines

Fattom *et al.* [23] at Nabi, Inc. (Boca Raton, FL) have prepared conjugates of CP5 and CP8 linked to rEPA that are intended for commercial use. The CP8-rEPA and CP5-rEPA conjugate vaccines were evaluated for safety and immunogenicity in 70 healthy adult volunteers [23]. Neither conjugate caused significant local or systemic reactions in the volunteers. The conjugate vaccines induced CP-specific antibodies of both the IgM and IgG classes. A second injection 6 weeks later did not have a booster effect. The authors suggested that the initial vaccination behaved more like a booster than a primary dose in these subjects because of their low levels of prevaccination capsular antibodies.

Nabi combined the CP5- and CP8-conjugate vaccines into a bivalent vaccine called StaphVAX that is intended for immunization of humans at high risk for *S. aureus* infection. They conducted a phase II, double-blinded, placebo-controlled clinical study of StaphVAX in approximately 230 patients receiving chronic ambulatory peritoneal dial-

ysis who were at high risk of staphylococcal disease. The patients were actively immunized with StaphVAX, and their antibody responses and infection rates were monitored. The vaccine elicited only mild local or systemic symptoms. The results of this trial indicated that the vaccine dose of 25 μ g of each CP was suboptimal in these patients. Their antibody responses to the vaccine were weak, and the immunized patients had infection rates similar to those of nonimmunized patients.

In subsequent phase II clinical trials, 32 volunteers with end-stage renal disease and 29 healthy controls were injected twice (6 weeks apart) with 25 μ g of CP5-rEPA or the bivalent conjugate vaccine (25 μ g each of CP5 and CP8 linked to rEPA). The vaccines elicited only mild local or systemic symptoms in both populations [27] (G Horwith, Personal communication). Four weeks after the second dose of vaccine, 23 of 24 healthy volunteers and 14 of 17 patients in one study responded to the immunization with a fourfold or greater increase in preimmunization levels of IgG and IgM antibody. However, the IgG and IgM levels of the patients were only approximately 50% of those achieved by the healthy controls at all postimmunization intervals. The monovalent and bivalent vaccines did not contain any adjuvant. Data from animal studies indicate that enhanced immunogenicity may be achieved by incorporating an adjuvant such as monophosphoryl lipid A [28].

Phase III clinical trial of the CP5/CP8/rEPA vaccine

Between 1998 and 2000, a phase III clinical trial to evaluate the efficacy of StaphVAX was conducted at the Kaiser Permanente Vaccine Study Center in California [29••]. This randomized, double-blinded, placebo-controlled study was designed to assess safety, immunogenicity, and ability to prevent bacteremia in patients with end-stage renal disease receiving hemodialysis. Approximately 1800

patients were enrolled in 73 hemodialysis centers. Half of the patients were administered a placebo, and the other half were immunized with Nabi's bivalent StaphVAX (100 µg each of CP5 and CP8 conjugated to an equal weight of rEPA). Patients receiving hemodialysis are at high risk for staphylococcal infection, with three to four of every 100 patients infected with *S. aureus* per year. In this clinical trial, the patients were monitored for culture-proven *S. aureus* bacteremia. There were no significant differences in the number of deaths in the vaccine and control groups, and none of the deaths were considered related to the vaccine. At the end of the study period (54 weeks), the vaccine reduced the incidence of bacteremia in the study population by only 26% (not significant). It was notable that the vaccine efficacy at 40 weeks was 57% ($P = 0.02$). However, after that time period, the antibody levels in the vaccinated patients declined, mirroring the decline in efficacy of the vaccine. StaphVAX was the first staphylococcal vaccine to reach clinical trials. Fattom *et al.* [29••] are hopeful that StaphVAX will provide better protection in other populations, such as patients who will undergo elective surgery and are at risk of systemic infection with *S. aureus*.

Passive immunization of humans with capsular antibodies

Another goal of Nabi, Inc., is to immunize healthy people with StaphVAX to generate an immunoglobulin product with high levels of antibodies to *S. aureus* CP5 and CP8. Polyclonal antibodies have been purified from the plasma of healthy vaccinees. In passive immunization studies, the IgG antibody generated in this fashion, referred to as StaphGAM, is administered to patients who are at immediate risk for staphylococcal infection. The circulating half-life of such antibodies is estimated to be 14 to 21 days [30]. Twenty-nine very low-birth-weight infants, who are at high risk for staphylococcal infection, were enrolled in a phase I/II safety and pharmacokinetics study conducted between 1998 and 1999 at the University of Texas. Subjects, who were stratified by weight (501–1000 g and 1001–1500 g), were administered two injections of AltaStaph 2 weeks apart. Three doses (500, 750, and 1000 µg/kg) of AltaStaph were administered, and the safety of each dose was assessed before escalation to the next dose level. All of the doses were well tolerated. Additional trials with AltaStaph are planned to test its efficacy as a prophylactic agent to prevent bacteremia. In addition, Nabi hopes to evaluate the therapeutic effect of AltaStaph as an adjunct to antibiotics in patients with documented *S. aureus* infection (G Horwith, Personal communication).

Poly-N-Acetyl Glucosamine (Polysaccharide Intercellular Adhesin)

McKenney *et al.* [31•] identified a new surface-associated polysaccharide on *S. aureus* that was poorly expressed in vitro but could be detected by serologic methods on organisms recovered from infections. The polysaccharide has

been given several names, including poly-N-succinyl β-1-6 glucosamine and polysaccharide intercellular adhesin [32]. The succinyl groups on the polymer have recently been shown to be an artifact of the acid hydrolysis protocol used to solubilize the polysaccharide for analysis by nuclear magnetic resonance. The correct structure is a variably N-acetylated polymer of β-1-6 glucosamine (G Pier, Personal communication). The protective efficacy of immunization with the glucosamine polymer was evaluated in a murine model of renal abscess formation. Mice immunized with three 100-µg doses of polysaccharide (5 to 6 days apart) before bacterial challenge showed significant reductions in CFU of *S. aureus* per gram of kidney compared with mice immunized with an irrelevant polysaccharide. Mice passively immunized with rabbit antibodies to the polymer before intravenous challenge with *S. aureus* and again 18 hours after challenge also were protected against renal infection and showed lower mortality than animals injected with antiserum to an irrelevant polysaccharide. Because a similar polysaccharide (called capsular polysaccharide/adhesin) is also elaborated by many isolates of coagulase-negative staphylococci, a vaccine to the β-1-6 glucosamine polysaccharide might target both coagulase-positive and coagulase-negative staphylococci. Previous studies showed that the polysaccharide adhesin was effective in laboratory animals as a vaccine against infections caused by coagulase-negative staphylococci [33,34].

Conclusions

Interest in the development of new approaches for the prevention of staphylococcal infections is a result of the worldwide dissemination of multiresistant strains of *S. aureus* and the increasing role of this microorganism in both nosocomial and community-acquired infections. The ideal vaccine against *S. aureus* would induce antibodies to prevent bacterial adherence, promote opsonophagocytic killing by leukocytes, and neutralize toxic exoproteins produced by the bacterium. Many impediments to the design of an effective staphylococcal vaccine exist, including the multitude of staphylococcal virulence determinants (*ie*, adhesins, exoenzymes, and exotoxins), and the complex pathways by which *S. aureus* regulates these factors. An evaluation of the experimental data from animal vaccine trials suggests that viable options for constituents of a multicomponent *S. aureus* vaccine would include one or more of the adhesins (fibronectin-binding protein, collagen-binding protein, and fibrinogen-binding protein [clumping factor]), a nontoxic alpha toxin mutant, CP5, and CP8. With the recent release to the public of the *S. aureus* genomic sequence and its ongoing analysis, it is likely that new targets for vaccine development will be unveiled and tested in the near future. Other avenues of investigation worth pursuing include the delivery of therapeutic agents such as the autoinducing peptides that target the regulatory networks that control expression of staphylococcal viru-

lence determinants. These agents and vaccines may be useful adjuncts to antibiotic therapy for *S. aureus* infections.

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