

# Progress Toward a *Staphylococcus aureus* Vaccine

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High attack rates and the ability of *Staphylococcus aureus* to develop resistance to all antibiotics in medical practice heightens the urgency for vaccine development. *S. aureus* causes many disease syndromes, including invasive disease, pneumonia, and skin and soft tissue infections. It remains unclear whether a single vaccine could protect against all of these. Vaccine composition is also challenging. Active immunization with conjugated types 5 and 8 capsular polysaccharides, an iron scavenging protein, *isdB*, and passive immunization against clumping factor A and lipoteichoic acid have all proven unsuccessful in clinical trials. Many experts advocate an approach using multiple antigens and have suggested that the right combination of antigens has not yet been identified. Others advocate that a successful vaccine will require antigens that work by multiple immunologic mechanisms. Targeting staphylococcal protein A and stimulating the T-helper 17 lymphocyte pathway have each received recent attention as alternative approaches to vaccination in addition to the more traditional identification of opsonophagocytic antibodies. Many questions remain as to how to successfully formulate a successful vaccine and to whom it should be deployed.

## PROBLEM OVERVIEW

*Staphylococcus aureus* is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), pneumonia, and invasive infections [1]. An epidemic of *S. aureus* infections with onset in the 1990s has intensified interest regarding this important pathogen [2]. In the United States, this epidemic has been driven by the serial emergence of 2 new *S. aureus* genetic backgrounds, USA400 and USA300, circulating in the community, predominantly as methicillin-resistant clones, so-called community-associated methicillin-resistant *S. aureus* (CA-MRSA) [3].

Available data suggest that *S. aureus* infections now constitute a public health imperative. Klevens et al [4] estimated that invasive MRSA infections occurred at a rate of 31.8/100 000 per year and were responsible for

the death of 18 650 patients (mortality rate: 6.3/100 000) in the United States in 2005. Liu et al [5] found that 1 in 316 people in San Francisco sought medical care for an MRSA infection in a recent year [5]. At Fort Benning, GA, it was recently estimated that the attack rate for medically attended MRSA infections was >35/1000 per year [6]. These data suggest an urgent need for improved strategies for control and prevention of *S. aureus* infections. They contrast with the much lower 2009 Centers for Disease Control and Prevention (CDC) case estimates of 0.28/100 000 with 0.04/100 000 mortality from meningococcal disease and 14.3/100 000 cases of invasive pneumococcal disease with a mortality rate of 1.6/100 000.

## ANTIBIOTIC RESISTANCE: A TALE OF REMARKABLE VERSATILITY

Resistant strains of *S. aureus* have been identified for every antibiotic introduced into clinical practice [7].

Resistance to vancomycin [8, 9], linezolid [10, 11], daptomycin [12], and mupirocin [13] have all been identified as clinical concerns. This continuing saga of antimicrobial resistance in *S. aureus* and the slowing of the development of new antimicrobials is reminiscent of similar clinical concerns with *Haemophilus influenzae* type b, where resistance to ampicillin and chloramphenicol, and *S. pneumoniae*, where resistance to penicillin,

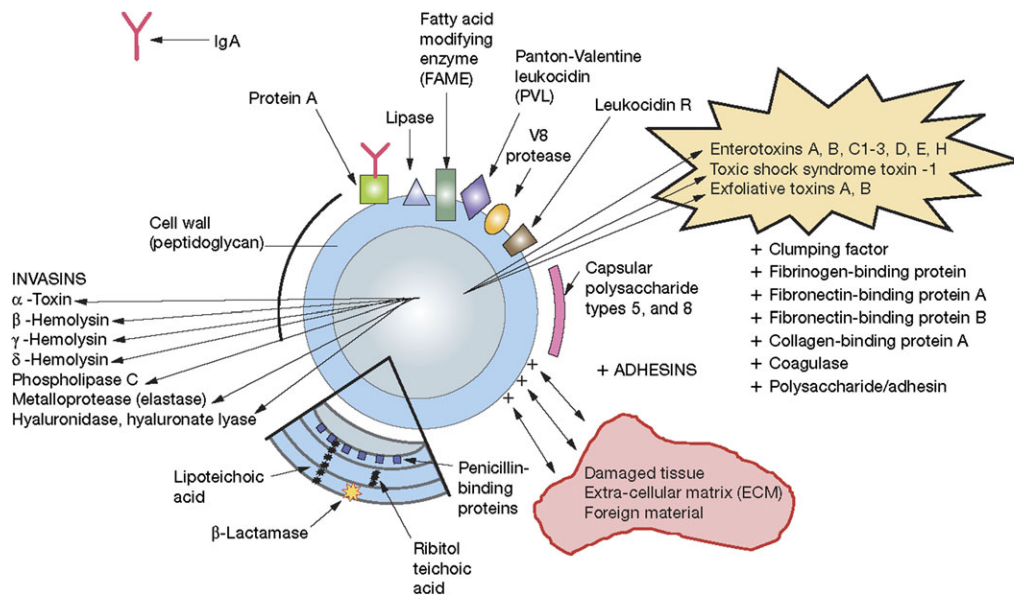
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**Figure 1.** Selected *Staphylococcus aureus* virulence factors. Abbreviation: Ig, immunoglobulin.

sounded clinical alarms that infections caused by these important pathogens had become increasingly difficult to treat. In both instances, the deployment of effective vaccination muffled many increasing concerns.

## CHANGING THE DEFINITION OF THE POPULATION AT RISK

If a vaccine against *S. aureus* were available, to whom would it be targeted? Prior to the late 1990s, MRSA infections occurred almost exclusively among patients with known exposure to the healthcare setting. However, the epidemic of CA-MRSA infections in the United States has required a redefinition of the risk factors for MRSA disease. The major change is that otherwise healthy individuals in the community are now at risk for MRSA infections [2]. Children, incarcerated populations, poor, homeless, young adults, military personnel in boot camps, day-care center contacts, household contacts, Pacific Islanders in Hawaii, Native Americans in Alaska, athletes (particularly those engaging in contact sports), patients with cystic fibrosis, and patients infected by human immunodeficiency virus (HIV) have all been affected, as have individuals who travel to or from the United States [2]. Many have been slow to grasp this profound change in MRSA epidemiology. Complexity has been added by several factors: HA-MRSA strains still circulate in the healthcare environment, although their transmission rate has decreased, likely due to improved infection control measures [14]. HA-MRSA isolates can sometimes be isolated from individuals in the community, especially adults; moreover, the new CA-MRSA strains have been detected in healthcare environments such as hospitals.

They can also be transmitted among community members who have healthcare risk factors. Also, methicillin-susceptible isolates of similar genetic background to CA-MRSA isolates also circulate in the community. Importantly, neither the term MRSA or methicillin-susceptible *S. aureus* designate a specific “strain” of *S. aureus*, and their use sometimes oversimplifies the complexity of the problem. Driven primarily by a large increase in USA300 infections, *S. aureus* infections have increased dramatically in the last 10–15 years. Therefore, a successful vaccine, the formulation of which has eluded researchers for many years, must be able to prevent disease caused by strains from a broad range of genetic backgrounds that possess a range of virulence factors and manifest in multiple clinical presentations.

## THE TARGET POPULATION

The increase in the clinical burden of *S. aureus* disease associated with the recent epidemic and the occurrence of many infections among previously healthy individuals has prompted discussions about whether there is a need for a universal immunization strategy or a niche-based one. Until now, development of a vaccine has proceeded slowly, in part because its use might be limited to the subpopulation at highest risk for invasive disease. Thus, active and passive immunization trials to date have been targeted to prevent, for example, bacteremia among dialysis patients [15], infections among neonates, or postoperative mediastinitis among patients undergoing elective cardiac surgery and sternotomy.

Why then contemplate a universal vaccine strategy? The attack rate for invasive *S. aureus* disease is high enough to

justify a universal approach. Both children and adults are at high risk [4]. If a single vaccine were effective against invasive disease, pneumonia, and SSTIs, the justification would be even greater. Such a vaccine could be conveniently integrated into the schedule for administration in the first year of life. Scheduled visits for immunizations during the first 6 months of life exist already into which a new vaccine could be incorporated. A goal would be to combine an effective *S. aureus* vaccine with other vaccine antigens such as a combination diphtheria/acellular pertussis/tetanus vaccine to make a new, combination vaccine. It is important to emphasize that, until the mid-1990s, identifying the subset of the population at risk for invasive *S. aureus* disease was relatively simple. However, the advent of the CA-MRSA epidemic has forced redefinition of the “at-risk” population. In advance of definitive definition of the “new” risk factors for CA-MRSA, one could argue that the entire population, given the correct exposure, should be considered at risk.

Other attempts to target subsets of the population at highest risk for disease have been unsuccessful. For example, hepatitis B vaccine became available in 1982 and initially was recommended only for certain high-risk subpopulations. However, this strategy failed to decrease the incidence of hepatitis B in the United States [16]. In contrast, a universal immunization strategy with 3 doses administered in early childhood has produced a marked decline in the incidence of hepatitis B in the United States [17, 18].

## **WHAT *S. AUREUS* INFECTIOUS SYNDROMES SHOULD A VACCINE PREVENT?**

An important issue concerns the expectations of the vaccine, should one be identified. *S. aureus* is a species well adapted to its human host. Most often, it is a commensal bacterium that can asymptotically colonize the nasopharynx, presumably by adhering to the nasal mucosa. The recent recognition that some *S. aureus* genetic backgrounds preferentially colonize the throat [19] (R. Daum et al, unpublished), rectum, or another site does not change the concept. Although it may be true that when bacteremia occurs, a colonizing, genetically identical strain can often be found in the nasopharynx [20], most colonized individuals do not become bacteremic. Furthermore, many individuals develop clinically apparent infection in the absence of detectable colonization.

Many patients with an SSTI do not seek medical attention. Despite this, SSTIs constitute the most frequent medically attended clinical illnesses, and constitute a major healthcare burden as many seek acute care in an emergency room or another facility. A few patients with an SSTI require hospitalization for major surgical drainage and/or intravenous antimicrobial therapy.

More serious invasive disease occurs in several settings. Otherwise healthy individuals in the community have sustained

severe, sometimes overwhelming infection. A few patients have been described with overwhelming sepsis with an illness whose tempo resembles meningococemia [21, 22, 23]. Others have had serious illness with a less fulminating course; examples include necrotizing pneumonia [24], osteomyelitis [25], and necrotizing fasciitis [26].

In addition to these disease syndromes occurring among otherwise healthy individuals, *S. aureus* causes serious infections among patients with underlying diseases such as diabetes mellitus and HIV. Additionally, it is well known that patients with indwelling intravenous access lines, or patients who receive hemodialysis, particularly when the integrity of the integument has been violated, are at high risk for *S. aureus* infections.

An ongoing discussion, then, concerns which clinical scenarios should be vaccine-preventable. Asymptomatic carriage, SSTI, pneumonia, or invasive disease are among the possibilities. Eradication of asymptomatic *S. aureus* carriage is probably not the appropriate primary goal for vaccine efficacy as many colonizing *S. aureus* genetic backgrounds are rarely isolated from medically attended disease. Prevention of SSTIs and pneumonia, on the other hand, would be desirable. Available evidence, however, from animal models suggests that the pathophysiology of these distinct clinical syndromes differs from each other and may require different immunomodulatory strategies for prevention. Invasive disease associated with bloodstream infection is the obvious target for vaccine efficacy. However, even here, it must be borne in mind that a bloodstream infection complicating an indwelling venous catheter most likely has different immunopathophysiology than a bloodstream infection in an otherwise healthy patient.

## **VACCINE DEVELOPMENT EFFORTS TO DATE**

How then to make the ideal *S. aureus* vaccine? It is increasingly clear that this will not be simple. There are several relevant considerations. It has been traditional to expect a vaccine to induce protective antibodies that will protect against infection caused by the target pathogen. One task, then, is to identify which is/are the protective antigens and which are the protective antibodies. *S. aureus* poses a challenge in this regard as many functions necessary for microbial pathogenicity, ranging from host cell adherence to iron scavenging (Figure), employ redundant microbial machinery; pathogenicity may not be interfered with if only 1 of several genes performing a similar function is targeted.

### **Cell Surface Polysaccharides as Vaccine Candidates**

Prior to the advent of epidemic USA300 disease in the United States, the identification of a polysaccharide capsule on the surface of most clinical *S. aureus* isolates suggested that they might prove to be effective vaccine antigens, particularly if conjugated to a protein carrier. After all, this approach proved successful in the

development of vaccines directed against the type b capsular polysaccharide of *H. influenzae*, multiple capsular polysaccharides of *S. pneumoniae*, and the capsular polysaccharides of serotypes A, C, W-135, and Y of *Neisseria meningitidis*. For *S. aureus*, 2 capsular types (5 and 8) comprised a majority of examined clinical isolate collections. Therefore, the polysaccharides from these 2 types were individually conjugated to a protein carrier, *Pseudomonas* exotoxin A. Efforts to show protection in animal models were hampered by variable capsule expression during growth and variable strategies for experimental challenge. Nevertheless, data were sufficiently encouraging to prompt a clinical trial sponsored by NABI Biopharmaceuticals. The target population was dialysis patients in northern California, and the clinical endpoint chosen for efficacy analysis was bacteremia.

The vaccine was well tolerated. The efficacy estimates from this trial provided a confusing picture. No significant efficacy was observed 2–20 weeks after immunization. Data analyzed at the 30- and 40-week postimmunization time points revealed modest efficacy (63%; 95% confidence interval [CI], 14%–86%, and 57%; 95% CI, 10%–81%, respectively). However, efficacy dissipated at the 50-, 54-, or 91-week postimmunization time points. At these times, the vaccine efficacy estimates were 25%–26% and insignificantly different from zero. Nevertheless, the investigators interpreted these data to indicate that the vaccine had provided some protection for about 40 weeks after immunization [15].

The geometric mean serum anticapsular antibody concentration among the vaccinees was 80 µg/mL, a value vastly exceeding concentrations believed to be protective against *H. influenzae* type b and *S. pneumoniae*. Additionally, there were no discernible differences between the peak antibody levels in bacteremic and nonbacteremic vaccinees. Moreover, the antibody concentrations peaked 6 weeks after immunization and declined thereafter, although the postulated significant protection was not observed until many weeks later.

About 20% of the blood isolates could not be assigned to type 5 or type 8 [15]. Although NABI claimed that these strains belonged to “serotype 336,” this has subsequently been shown to be wall teichoic acid [27], present in both capsular-positive and -negative isolates.

A second randomized placebo-controlled clinical trial with this bivalent capsular polysaccharide-protein conjugate vaccine was conducted at ~200 sites in the United States, also among dialysis recipients. Efficacy was evaluated 3–35 weeks post-immunization. This trial produced a clearer result in that there was no decrease in types 5 and 8 *S. aureus* bacteremia among vaccinees (<http://www.bizjournals.com/southflorida/stories/2005/10/31/daily27.html>, accessed November 2011).

Why did the types 5 and 8 conjugated capsular polysaccharide vaccine prove unsuccessful? There are several possible explanations. Most simply, the capsule may not be the protective antigen. Unlike the case with *H. influenzae* type b and

*S. pneumoniae*, where it was clear that circulating anticapsular antibody was protective, no such demonstration has been made for *S. aureus*. Second, many strains are unencapsulated. A relatively high frequency of isolates lacking the types 5 and 8 capsule was found in the clinical trial among dialysis patients. Importantly, USA300, the genetic background most responsible for the CA-MRSA outbreak currently affecting the United States, does not elaborate either the type 5 or type 8 capsule [29]. Third, it is possible, although unlikely, that the capsules are protective antigens but that dialysis patients were too immunocompromised to be protected against *S. aureus* bacteremia by this antibody-mediated approach.

Another carbohydrate antigen, poly-N-acetylglucosamine (PNAG), is a surface polymer produced by a variety of bacterial species, including *S. aureus* and *S. epidermidis* [30]. PNAG is an adhesin that facilitates bacterial cell-to-cell contact in biofilms [31]. Its biosynthesis is controlled by a locus called *icaADBC* [32]. PNAG exists in acetylated and deacetylated forms [33]. The latter is retained on the staphylococcal cell surface [33, 34]. Interestingly, antibodies to the acetylated and the deacetylated forms differ in their biologic activity. Only antibodies to the deacetylated PNAG are opsonic [35]. When rabbits were immunized with a deacetylated conjugate PNAG vaccine, their serum, passively administered to mice, evoked a modest decrease in bacteremia inoculated with 1 *S. aureus* strain. Serum samples raised to a nondeacetylated PNAG conjugate did not decrease bacteremia in the 2 strains tested. Thus, deacetylated PNAG (dPNAG) but not PNAG may be a protective antigen against *S. aureus* invasive disease [36]. A monoclonal antibody prepared against dPNAG has been prepared for a clinical trial, although the target population has not yet been identified.

### ***S. aureus* Proteins as Vaccine Candidates**

Efforts are underway to search for other protective antigens. Kuklin et al [37] at Merck identified the Fe-scavenging, cell wall-anchored protein *isdB* as a potential vaccine candidate. It was identified because patients with *S. aureus* infections had a brisk antibody response to it upon convalescence. The protein proved immunogenic in mice and rhesus monkeys. A phase II/III trial among adults scheduled for elective cardiac surgery was underway at multiple medical centers with an endpoint of protection against *S. aureus* mediastinitis. However, enrollment was halted in April 2011 for reasons that have not yet been made public (Table 1).

Adhesins such as clumping factor A (ClfA) have also received attention as possible protective antigens. ClfA binds to fibrinogen, blood clots, damaged epithelium, and platelets, often important early targets in *S. aureus* pathogenesis. It has been hypothesized that antibody limiting *S. aureus* from performing these tasks might negatively impact its invasiveness. Accordingly, Veronate, an immunoglobulin preparation made from a pool of high-titer anti-ClfA serum samples, was administered to neonates,

**Table 1. S. aureus Vaccines Receiving Clinical Evaluation As of November 2011**

Enrolling or about to enroll			
Manufacturer	Vaccine	Type of Study	Composition of Vaccine
Novartis	4 component	Phase I-Adult	Not publicly disclosed. Components are all proteins
Pfizer	4 component	Phase-I-Adult	Conjugated capsular polysaccharides, types 5 and 8, Clumping factor A, Manganese transporter C
Novadigm	recombinant protein	Phase-I-Adult	rA13p-N
GSK	4 component	Phase-I-Adult	Not publicly disclosed
Enrollment Complete			
Merck	1 component	Phase II/III efficacy	isdB <sup>1</sup>
Biosynexus	Passive monoclonal antibody	Phase IIb/III efficacy	anti-lipoteichoic acid antibody <sup>2</sup>

<sup>1</sup> Enrollment halted 2011 by recommendation of the Independent Data Monitoring Committee.

<sup>2</sup> No significant decrease in 'staphylococcal sepsis' among very low birth weight neonates.

a high-risk population for invasive *S. aureus* disease. Despite some promise from an initial trial [38], a phase III trial aimed at preventing *S. aureus* bacteremia among neonates was not successful [38]. Additionally, a monoclonal anticlumping factor A antibody called tefibazumab (Aurexis) (<http://en.wikipedia.org/wiki/Tefibazumab>, accessed November 2011) was given to adults with *S. aureus* bacteremia with some demonstrated efficacy against relapse and complications of bacteremia [39]. The next steps for Aurexis have not yet been made public.

Other protein antigens have received preclinical evaluation. The antigens include the Pantone-Valentine leukocidin [40],  $\alpha$ -hemolysin [41], and a vaccine containing isdA, isdB, SdrD, and SdrE [42].

#### Other Vaccine Candidate Antigens

The notion that lipoteichoic acid might generate protective antibody has received recent attention. Indeed, a monoclonal antibody against lipoteichoic acid called pagibaximab was in clinical trial among neonates with the stated goal of preventing *S. aureus* and coagulase negative staphylococcal bacteremia. Enrollment was completed in 2010, but "no significant decrease in staphylococcal sepsis" was found.

Other vaccines are under study in early clinical trials sponsored by Pfizer, Novartis, Novadigm, and GSK. Their composition, if publicly revealed, is presented in the Table.

#### WHAT ABOUT LOW-LEVEL ANTIBODIES FOUND IN HUMAN SERUM SAMPLE SURVEYS?

A poorly understood conundrum concerns the presence of "low-level" antibody to many staphylococcal proteins in nonimmune human serum. Antibodies to putative protective antigens such as the Pantone-Valentine leukocidin,  $\alpha$ -toxin ( $\alpha$ -hemolysin), or ClfA and many other 'virulence factors' are widely prevalent. Their role is not clear. Some have suggested that these antibodies do not have the necessary functional activity for protection. Others have

suggested that low-level antibodies such as these cannot overcome ineffectual protein A binding. Still others have suggested that there is differential susceptibility to *S. aureus* infection and individuals with low-level antibodies targeted to protective antigens are not the population at risk.

#### DEALING WITH PROTEIN A

Protein A is a 40–60 kDa signature cell-wall protein encoded by the *spa* gene. It is synthesized by nearly all *S. aureus* isolates and binds to the Fc region of human immunoglobulin (Ig) G1 and IgG2 with high affinity as well as mouse IgG2a and IgG2b. Binding of protein A to the Fc  $\gamma$  region of the immunoglobulin molecule through interaction with the heavy chain results in the wrong orientation (with respect to physiologic antibody function), and thus protein A could interfere with the role of antibody in opsonization and phagocytosis. Protein A may therefore protect *S. aureus* from antibody attack. Protein A has also been termed a B-cell superantigen [43], because it also binds to V<sub>H</sub>3 B-cell receptors and limits their antibody manufacturing capacity. Furthermore, marginal zone B cells and B-1 cells undergo, preferentially, induced cell death with supraclonal depletion and immune tolerance upon exposure to protein A. Thus, protein A may limit the efficacy of a vaccine that depends on opsonophagocytic antibody production. Protein A might also interfere with vaccine efficacy by virtue of B-cell binding and interfering with B-cell function.

Kim et al [44] recently engineered a mutant protein A molecule that had mutations targeted to the immunoglobulin binding site and the V<sub>H</sub>3 B-cell binding site. This mutant protein A SpA<sub>KKAA</sub> molecule does not bind the Fc fragment of immunoglobulins or trigger B-cell apoptosis. Immunization of mice with SpA<sub>KKAA</sub> elicited antibodies that neutralized immunoglobulin-binding activities of protein A, provided some protection in a mouse model of bacteremic *S. aureus* infection, and augmented immunogenicity of unrelated antigens.



## THE ROLE OF T CELLS

A new chapter in *S. aureus* vaccine development was recently opened by the recognition that lymphocyte-mediated pathways play a role in immunity to *S. aureus* infections. Several observations increase the plausibility of this idea. Spellberg et al [45] found that interferon  $\gamma$  (IFN- $\gamma$ )-deficient mice were hypersusceptible to infection when *S. aureus* was inoculated intravenously [45–47]. Others found that mice deficient in interleukin 17A (IL-17A) and F (IL-17F) developed spontaneous *S. aureus* skin infections [48, 49]. Hence, it may be possible to induce memory T cells that are capable of increasing phagocyte recruitment to sites of infection and facilitate clearance of *S. aureus* from tissues. In support of this, a novel vaccine strategy against *S. aureus* has been based on the immunologic activity of a candidal adhesin rAls3p. A recombinant N-terminus vaccine molecule, rAls3p-N, is under investigation as a putative vaccine candidate [45–47]. The rAls3p-N vaccine induced high antibody concentrations, but these antibodies were not protective when used to passively immunize against *S. aureus* intravenous challenge [45]. Serum concentrations of anti-rAls3p-N antibodies in individual mice did not correlate with the risk of death from *S. aureus* infection [47]. Furthermore, the vaccine was equally effective in B-cell-deficient and wild-type mice but had no efficacy in T-cell-deficient mice [45]. Adoptive transfer of immune B220<sup>+</sup> B cells did not transfer protection, but transfer of CD4<sup>+</sup> T cells did. The vaccine was ineffective in IFN- $\gamma$ - and IL-17A-deficient mice and in gp91<sup>phox-/-</sup> mice that are unable to produce superoxide [47]. Cross-adoptive transfer experiments confirmed that functional phagocytes were operative in vaccine-mediated protection at the downstream effector stage. Additionally, vaccination increased the recruitment and activation of phagocytes at sites of tissue infection in mice, and cytokines produced by vaccine-primed lymphocyte improved the ability of phagocytes to kill *S. aureus*. Hence, the rAls3p-N vaccine demonstrates that it is feasible to induce a protective immune response in mice against *S. aureus* in the absence of induction of protective antibodies and by inducing a protective T-helper 1 (Th1)/Th17 response [50–52].

These observations do not preclude development of a humoral-based vaccine against *S. aureus*. Rather, they suggest that cell-mediated vaccines merit additional focus and raise the possibility of combining antigens that stimulate humoral and cellular responses against *S. aureus*. Indeed, this strategy may be the most likely to result in a protective vaccine against *S. aureus*.

## CONCLUSION

How should a *S. aureus* vaccine be formulated? The traditional approach of identifying the correct antigen or antigen combination and relying on the production of protective antibody has come under recent scrutiny and may need to be modified.

Although many experts believe that multiple vaccine antigens should be included in a vaccine formulation to prevent *S. aureus* infections, this view is usually advocated to mean that multiple antigens that elicit multiple antibodies that together offer protection will be required.

What about capitalizing on new concepts recently learned? Perhaps the notion that multiple antigens will be required can be reformulated to mean that multiple immunologic approaches will be required, such as the blunting of interference from protein A and the stimulation of the Th17 pathway. It is possible, then, that the optimal strategy should employ multiple antigens that work by multiple immunologic mechanisms. Whether such an approach can be explored and would prove superior to those currently under investigation will require additional study.

How could an efficacy/effectiveness trial be designed? This is not an easy question, and a full discussion is beyond the scope of this review. Examples of possible relevant target populations include (1) military recruits, (2) household contacts of index patients, and (3) patients with a single episode of *S. aureus* infection who have a high rate of recurrent disease. All 3 of these populations have relative ease of ascertainment and suitably high attack rates, thus allowing calculation of a feasible sample size.

## Notes

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