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REVIEW

Development of a vaccine against *Staphylococcus aureus*

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Abstract A vaccine to prevent infections caused by *Staphylococcus aureus* would have a tremendously beneficial impact on public health. In contrast to typical encapsulated bacterial pathogens, such as *Streptococcus pneumoniae*, *H. influenzae*, and *Neisseria meningitides*, the capsule of *S. aureus* is not clearly linked to strain virulence in vivo. Furthermore, it is not clear that natural infection caused by *S. aureus* induces a protective humoral immune response, as does infection caused by typical encapsulated bacteria. Finally, pure B cell or antibody deficiency, in either animal models or in patients, does not predispose to more frequent or more severe *S. aureus* infections, as it does for infections caused by typical encapsulated bacteria. Rather, primary immune mechanisms necessary for protection against *S. aureus* infections include professional phagocytes and T lymphocytes (Th17 cells, in particular) which upregulate phagocytic activity. Thus, it is not clear whether an antibody-mediated neutralization of *S. aureus* virulence factors should be the goal of vaccination. Rather, the selection of antigenic targets which induce potent T cell immune responses that react to the broadest possible array of *S. aureus* strains should be the focus of antigen selection. Of particular promise is the potential to select antigens which induce both humoral and T cell-mediated immunity in order to generate immune synergy against *S. aureus* infections. A single-antigen vaccine may achieve this immune synergy. However, multivalent antigens may be more likely to induce both humoral and T cell immunity and to induce protection against a broader array of *S. aureus* isolates. A number of candidate vaccines are in development, raising the promise that effective vaccines against *S. aureus* will become available in the not-so-distant future. Possible development programs for such vaccines are discussed.

Keywords *S. aureus* · Vaccine · Development · Immunology

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Introduction

Staphylococcus aureus is a ubiquitous pathogen. It is the most common cause of culture-confirmed skin and soft tissue infections (SSTIs) [1–4] and endocarditis [5], and is the second most common cause of bacteremia [6, 7]. *S. aureus* is also a predominant cause of a variety of nosocomial infections, including ventilator-associated pneumonia, intravenous catheter-associated infections, postsurgical wound infections, as well as invasive infec-

tions in neutropenic patients and in patients undergoing solid organ or hematopoeitic stem cell transplants [8].

Invasive infections caused by *S. aureus* continue to increase in frequency [9, 10]. Population-based estimates of the incidence of *S. aureus* infections have ranged from a low of approximately 30 per 100,000 [11, 12] for analyses restricted to invasive (beyond skin) disease to up to 600 per 100,000 based on extrapolation from a surveillance study in the USA [13]. In a separate study from Europe, the incidence of just bacteremia caused by *S. aureus* was reported to be 30 per 100,000 [14]. Given that >90% of infections caused by *S. aureus* are skin infections [15], incidences of *S. aureus* bacteremia of ~30 per 100,000 support the estimate of approximately 600 per 100,000 total incidence of *S. aureus* disease reported by the CDC. Therefore, there may be 1.8 million cases of *S. aureus* infection (including skin infection) per year in the USA alone, which obviously provides a potentially massive market to stimulate interest in vaccine development.

Community-associated *S. aureus* infections were formerly nearly uniformly susceptible to penicillinase-resistant beta lactams (i.e., methicillin, oxacillin, etc.). However, over the past decade, a number of community-based outbreaks of methicillin-resistant *S. aureus* (MRSA) infections have emerged, initially in pediatric populations [16–18]. Community outbreaks of MRSA infections also have been seen in adults in multiple locales in the USA and across the globe, and in many places, MRSA has become the predominant *S. aureus* strain causing community-acquired infections [15, 19–23]. The rise in MRSA incidence underscores the need to develop new strategies to prevent invasive *S. aureus* infections.

Given its high incidence of causing life-threatening, drug-resistant infections, a vaccine to prevent *S. aureus* infections would have an enormous and beneficial impact on global and US health. The purpose of this review was to broadly discuss challenges to and the promise of developing *S. aureus* active vaccines. Issues ranging from fundamental immunology, to antigen targets, to practical considerations for development programs are discussed.

Fundamental immune underpinnings of a *S. aureus* vaccine

What type of immune response should be stimulated?

When rationally designing a vaccine against a specific disease, it is desirable to understand what mode of immunity, when induced, will serve to protect the host from the target infection. Vaccines which induce the protective mode of immunity can then be developed. There are two types of studies which can elucidate the nature of protective immunity against specific diseases. For example, natural infection may induce a specific type of memory immune response which protects against subsequent reinfection. Identification of the nature of that protective memory response in patients with previous natural infection can be used to elucidate what type of immune response to focus on when vaccinating immunologically naive patients.

A second means to elucidate the nature of protective immunity against a specific disease is definition of the nature of immune defects which specifically predispose to the disease. These two types of data—description of the type of immunity that is protective after natural infection and description of specific immune defects that predispose to infection—provide complementary information. Specifically, protective immunity after natural infection elucidates immunity that is sufficient to protect against reinfection, and immune defects that predispose to infection elucidate immunity that is necessary to protect against infection.

Based on these principles, the design of vaccines targeting toxin-mediated diseases is relatively straightforward: immunize with a detoxified toxin analogue to generate neutralizing antibodies (e.g., diphtheria, pertussis, tetanus toxoid). Similarly, for viral diseases, successful vaccines based on whole virus (e.g., hepatitis A virus), viral antigens (e.g., hepatitis B virus), or viral-like particles (e.g., human papilloma virus) have been designed to generate antibodies that block viral interaction with host cells and induce cytotoxic T lymphocytes to kill virally infected host cells [24]. In these examples, the vaccines are designed to induce an immune response which mimics protective immunity against natural disease caused by the target toxin or pathogen and also stimulates mechanisms of host defense which, when absent, predispose to the target disease.

Similarly, the underpinning of vaccination against typical encapsulated bacterial pathogens (e.g., *Streptococcus pneumoniae*, *Hemophilus influenzae* type b, *Neisseria meningitidis*) is based in part on the well-described hypersusceptibility to these infections of patients with congenital or acquired B cell/antibody deficiencies as well as the same hypersusceptibility of animal models in which B cell/antibody function is disrupted [25]. These clinical and experimental immunologic observations are further bolstered by data establishing that antibody concentrations correlate with protective immunity after natural infection caused by such encapsulated organisms [24]. Hence, vaccines against encapsulated bacterial pathogens are designed to stimulate antibodies that neutralize the antiphagocytic capacity of the polysaccharide capsule of *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, enabling the host immune system to clear the organism [24].

After more than a half century of successful vaccine development for toxin, viral, and encapsulated bacterial

diseases, it is perhaps not surprising that initial efforts to develop a staphylococcal vaccine were based on a presumption that the same immunologic mechanism would be protective: a humorally focused vaccine and one that targeted capsular polysaccharide just as for other bacterial pathogens. Unfortunately, efforts to date to develop such a vaccine have not been successful (discussed further below). A primary reason for such efforts not having been successful is likely that the fundamental immunopathogenesis of *S. aureus* infections differ from the immunology of toxin-based, viral, and typical encapsulated bacterial infections.

S. aureus immunology and immunopathogenesis

The nature of protective immunity sufficient to protect against reinfection following natural infection is not known for *S. aureus*

Unfortunately, in contrast to toxin, viral, and encapsulated bacterial infections, we have virtually no understanding of the correlates of protective immunity against *S. aureus* infection. It is clear that *S. aureus* invasive infections result in the generation of a memory immune response typified by specific antibody at higher titers post-infection than pre-infection [26–30]. However, it has not been established that the memory immune response occurring after natural infection with *S. aureus* results in protection against reinfection. One recent study found no relationship between specific anti-Panton Valentin leukocidin (PVL) antibody titers post-infection and risk of recurrence [28]. Furthermore, recurrence is a well-established clinical hallmark of *S. aureus* cutaneous abscesses [31, 32]. Indeed, in various studies analyzing widely disparate patient populations, approximately 10–30% of initial *S. aureus* cutaneous abscesses resulted in recurrence [33–39]. Hence, natural infection with *S. aureus* does not reliably result in an immune response which prevents future recurrence. Absent knowing that a protective memory response occurs after natural *S. aureus* infection, it is certainly not possible to define the nature of such protective memory responses.

Much has been published regarding the ability of *S. aureus* to subvert normal host defense mechanisms [40, 41]. Mechanisms of the organism implicated to achieve such immune subversion include the prevention of complement deposition, prevention of antibody-mediated opsonophagocytosis, evasion of phagocytic killing, toxin-mediated lysis of white blood cells, and dysregulated immune hyperactivation via superantigens. None of these mechanisms clearly affect the generation of a memory response to infection or elucidate why such responses would or would not be protective.

Indeed, clinical experience suggests that protective immunity may actually develop post-infection in some patients, although it likely does so variably for reasons that we do not understand. For example, while recurrent infections are common in patients with *S. aureus* cutaneous abscesses, many patients never develop recurrences despite the ubiquity of the pathogen. Furthermore, for those who do develop recurrences, eventually, even repeated recurrences typically cease.

More compelling regarding the role of adaptive cell-mediated immunity in protecting against recurrent *S. aureus* infections is the fact that patients with defects in T cell immunity (e.g., HIV) clearly have higher recurrence rates than patients with intact T cell function; 50–70% of HIV-infected patients with an initial *S. aureus* abscess develop recurrence [39, 42–44], which is approximately two- to threefold higher than the previously discussed recurrence rate in non-HIV-infected patients. Similarly, as discussed further below, patients with genetic defects in STAT3, resulting in hyper-IgE syndrome (also called Job's syndrome) and defective Th17 cell function, experience recurrent and often severe *S. aureus* abscesses [45–49]. These results strongly suggest that an intact cell-mediated immune axis results in immunological memory that reduces the risk of recurrence of *S. aureus* infections compared with patients with defective cell-mediated immunity. Furthermore, as mentioned, many patients never develop recurrence of cutaneous abscesses, suggesting that immunity may develop in some patients after initial cutaneous infection. Why protective memory immunity may develop post-infection with *S. aureus* in some patients and not in others is not clear, and immunological differences which distinguish protective from non-protective responses have never been elucidated.

Hence, in contrast to diseases for which vaccines have been successfully deployed (i.e., toxin-mediated, viral, and typical encapsulated bacteria), we simply do not know the correlates of protective immunity after natural infection for *S. aureus*. This absence of understanding of immune correlates of protection greatly complicates preclinical selection and optimization of vaccine candidates, and further complicates clinical development, since surrogate markers for protection *cannot be known* during phase I and II clinical trials. Quite simply, until a vaccine against *S. aureus* is shown in the clinic to prevent infection caused by *S. aureus*, only clinical outcome data can be used to evaluate the efficacy of a *S. aureus* vaccine. Once an effective vaccine is developed, immunological markers can be compared as to their efficacy to determine which markers serve as correlates of protective immunity. Then and only then can such markers serve to reliably focus and simplify the preclinical selection and clinical development of subsequent *S. aureus* vaccine candidates.

Critical lessons from available natural experiments: what type of host defense mechanism(s) is necessary to protect against *S. aureus* infections?

It is clearly established that patients deficient in B lymphocytes or with congenital or acquired deficiency in specific antibody production are at a markedly higher risk of acquiring infections caused by typical encapsulated bacteria and a variety of viral infections [50–52]. These infections are not only more common but also typically far more severe when they occur in B lymphocyte- or antibody-deficient patients. Hence, not only are antibodies sufficient for protection against such infections (because they result in natural immunity post-infection) but they also appear to be necessary to protect against such infections.

What do similar natural experiments tell us regarding *S. aureus* infections? The immunopathogenesis of *S. aureus* infections stands in contrast to typical encapsulated bacterial infections. Patients with pure B cell or antibody deficiency, or asplenic states, are not at a higher risk of *S. aureus* infections, nor do they have especially severe *S. aureus* infections when such infections occur [25]. The exception that proves the rule is that antibody-deficient patients with recurrent respiratory tract infections that lead to anatomical abnormalities (e.g., loss of normal ciliary function, bronchiectesis, lung blebs) are at increased risk of *S. aureus* superinfections [51, 52]. These infections do not occur until later in life, after patients have experienced sufficient numbers of upper respiratory infections caused by encapsulated bacterial pathogens to cause the anatomical abnormalities. Hence, these *S. aureus* infections are the result of the anatomical abnormalities, not the antibody deficiency per se. Thus, in contrast to viral infections and encapsulated bacterial infections, B cells and antibody are not necessary for host defense against *S. aureus* infections.

Patients who are definitively at increased risk of developing *S. aureus* infections are those with disruptions of anatomical barriers (e.g., by cutaneous burns, intravenous catheters, endotracheal tubes, surgical or traumatic wounds, post-viral ciliary airway disruption) and those with quantitative (e.g., chemotherapy-induced neutropenia) or qualitative (e.g., chronic granulomatous disease, leukocyte adhesion deficiency, Chediak–Higashi syndrome) neutrophil disorders.

As mentioned, the increased risk of *S. aureus* infections in HIV-infected patients supports the concept that T cell dysfunction is a risk factor for such infections. Furthermore, the role of T cells in host defense against *S. aureus* infections is supported by the recent elucidation that STAT3 deficiency results in hyper-IgE syndrome [45–49]. STAT3 is a critical upstream regulator of Th17 differentiation, and Th17 dysfunction or absence is a prominent immunological phenotype in hyper-IgE syndrome. Hyper-IgE syndrome patients and those with Th17 defects have a markedly increased risk in developing both cutaneous and pulmonary infections caused by *S. aureus*. These findings provide a critical clue to the link between T cell function and neutrophil function in host defense against *S. aureus* infections. Specifically, Th17 cells are potent inducers of neutrophil chemotaxis to sites of infection and enhance the activation of neutrophils at sites of infection [53, 54]. Disruption of the Th17 axis therefore results in a delayed, diminished recruitment of neutrophils and a diminished activation of the phagocytes, which facilitate long-term bacterial persistence and chronic inflammation at sites of infection.

Recently, murine models have been described to recapitulate, in well-controlled experiments, the above clinical findings. Specifically, we reported for the first time that B cell-deficient mice are no more susceptible to systemic/bloodstream infection caused by *S. aureus* compared with wild-type mice [55]. In contrast, T cell-deficient mice were hypersusceptible to infection. $gp91^{phox-/-}$ superoxide-deficient mice (a model of chronic granulomatous disease) were also hypersusceptible to infection. Furthermore, Th17 cells were necessary for vaccine-induced protection against *S. aureus* infection in the murine model, and such cells acted by enhancing neutrophil recruitment to sites of infection and by enhancing killing of *S. aureus* by neutrophils [56].

We have also found that IFN-γ-deficient mice are hypersusceptible to infection caused by *S. aureus* inoculated intravenously [55, 57]. Others have found that dual IL-17A/F-deficient mice had an increased incidence of developing spontaneous skin infections caused by *S. aureus* [58]. Hence, a new immunologic strategy to develop an anti-*S. aureus* vaccine may be to induce memory T cells which are capable of increasing the rapidity and strength of phagocyte recruitment to sites of infection, facilitating clearance of the organism from tissues.

In contrast to the systemic model of infection, in the skin model of infection, B cell deficiency resulted in the exacerbation of the size of the dermonecrotic lesion, whereas T cell deficiency and IL-17A deficiency resulted in smaller dermonecrotic lesions (manuscript submitted). The impact of lymphocyte and IL-17A deficiency on lesion size was due to alterations in inflammatory response, and lesion size correlated better with inflammatory cytokine and neutrophil influx levels than with tissue bacterial burden. Hence, the size of cutaneous infections appears to be driven as much by the inflammatory response to the organism as by the number of organisms present. Vaccination resulted in a more balanced Th1–Th2 inflammatory response while still enhancing neutrophil influx, leading to the amelioration of lesion size.

Thus, clinical experience with patients with congenital or acquired immune defects combined with data from carefully controlled murine models indicates that antibodies are not required for host defense against *S. aureus* infections. In contrast, normal anatomical barriers to infection and normal numbers and functions of neutrophils are critical to host defense against *S. aureus* infections. T cells play a critical role in regulating the downstream phagocytic response, both by enhancing the recruitment and activation of neutrophils to better kill *S. aureus* at sites of infection and also possibly by restraining out-of-control inflammation in cutaneous lesions. These data do not support the concept that vaccination against *S. aureus* should be designed to stimulate the same type of immunity as for toxin, viral, or encapsulated bacterial infections.

A review of past and present *S. aureus* vaccines: focus on antigen selection

Capsular polysaccharide vaccines

For many years, efforts to develop an anti-*S. aureus* vaccine candidate were focused on capsular polysaccharide as a putative protective antigen. The leading effort in this regard was StaphVAX, a bivalent vaccine comprising *S. aureus* capsular polysaccharide types 5 and 8 bound to pseudomonal exotoxoid A as a carrier. In phase II clinical trials, the vaccine resulted in high antibody titers that lasted for approximately 6 months in patients undergoing chronic hemodialysis [59–61]. Furthermore, a booster dose appeared to maintain antibody levels for more than a year. Unfortunately, in a large pivotal phase III trial, StaphVAX did not reduce the incidence of invasive *S. aureus* infections in hemodialysis patients [62]. This lack of protective efficacy occurred despite the presence of impressive, opsonophagocytic, anti-capsular antibody concentrations in immunized patients. Thus, the induction of specific immune serum to *S. aureus* does not necessarily result in protection, and anti-capsular antibodies are not inherently protective against *S. aureus* as they are against, for example, *S. pneumoniae*.

Aside from the immunological concerns regarding the role of B cells in host defense against *S. aureus*, another factor limiting a capsular polysaccharide approach is that many clinical isolates lack a capsule. For example, the major genetic background causing epidemic community-acquired MRSA infection, USA300, elaborates no detectable capsular polysaccharide [63]. Furthermore, in the phase II trial of StaphVax, 20% of isolates were identified as having type 336 capsular antigen [61], which has subsequently been reported to be polyribitol phosphate *N*-acetylglucosamine (resembles cell wall teichoic acid) and is not a capsular polysaccharide at all [64, 65]—that is, 20% of isolates in the trial had no detectable capsule.

Surface antigen passive immunization

Passive vaccine strategies targeting *S. aureus* surface antigens have also been attempted. The Aurexis(™) anti-staphylococcal monoclonal antibody targets the microbial surface components recognizing adhesive matrix molecule, clumping factor A. A phase II clinical trial of Aurexis(™) as an adjunctive therapy in patients with established *S. aureus* bacteremia resulted in a non-significant trend toward improved outcomes for treated patients [66]. However, in another study, high-titer anti-clumping factor A polyclonal antibody resulted in no clinical benefit among high-risk premature neonates and did not reduce the risk of developing invasive staphylococcal infection [67]. Another surface target is wall techoic acid. Biosynexis has a chimeric monoclonal antibody targeting wall techoic acid in phase III clinical trials for the prevention of staphylococcus sepsis in very low birth neonates (NCT00646399). The results of the trial are anticipated in 2011.

The failure of an active, polysaccharide capsular-based vaccine despite a successful induction of opsonophagocytic antibodies, combined with the failure of passive immunization against *S. aureus* surface proteins in clinical trials, highlights a logical disconnect between these humoral-based strategies deployed against *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* versus a similar approach against *S. aureus*.

Virulence factors as target antigens

Another strategy to develop a *S. aureus* vaccine has been based on the notion that the neutralization of virulence factors elaborated by the bacterium could ameliorate or prevent disease. Numerous virulence factors have been targeted by vaccination, including alpha hemolysin, PVL, clumping factor A, fibrinogen binding protein, enolase (laminin-binding protein), and protein A. A recent study found that antibodies against LukD and LukF (leukocidins), alpha toxin, and SEA (in addition to antibodies against numerous other antigens) were generated after *S. aureus* bacteremia in mice [68]. Different vaccines targeting such antigens have been shown to provide varying levels of protection to mice against systemic infection caused by *S. aureus*. For example, active vaccination with a detoxified alpha hemolysin and passive immunization against alpha hemolysin ameliorated the size of skin lesions and the severity of pneumonia in mice infected with a USA300 strain of MRSA [69–71]. Vaccination with PVL subunits also ameliorated the severity of pneumonia and skin infections in mice infected with MRSA [72]. A variety of

adhesins, including clumping factor A, clumping factor B, enolase, and fibrinogen-binding protein, have all been shown to ameliorate the severity of systemic *S. aureus* infection or prevent nasal colonization by *S. aureus* in mice [73–79].

Some of these vaccine candidates have thus far only been shown to be effective when administered with Freund's adjuvant, which is too toxic for clinical use. Similarly, when administered with Freund's adjuvant, a vaccine containing multiple antigens was more effective than a single-antigen vaccine against IV infection [80]. The enhanced efficacy of a multi-antigen vaccine in this study has become important to the belief that virulence factor neutralization can be an effective strategy for a *S. aureus* vaccine, but that multiple factors must be neutralized by vaccination because *S. aureus* elaborates so many factors that contribute to its pathogenesis [77, 80–82]. Working on this hypothesis, a pentastaph vaccine containing capsular polysaccharide types 5 and 8, techoic acid (cell wall antigen type 336), Panton Valentin leukocidin, and alpha hemolysin has been developed initially by Nabi and then acquired by Glaxo Smith Kline. That vaccine is currently in phase I clinical trials (NCT01160172).

It must be emphasized that an antibody-mediated neutralization of virulence factors has never been established to be an effective mechanism of protection against *S. aureus* infection. It is not at all clear that virulence factors, whether toxins or surface adhesins, make better antigens than any other types of targets which induce potent immune responses. Nevertheless, it is possible that a multivalent vaccine will result in superior efficacy because it could facilitate the development of a vaccine that: (1) results in the induction of both humoral and cell-mediated immunity by distinct antigens and (2) results in a greater probability that any one antigen included will trigger a potent immunodominant response in highly genetically diverse populations since both mice and humans have extremely variable antibody responses to a diverse array of antigens from *S. aureus* post-infection [29, 68]. Furthermore, it is clear that different anatomical sites of infection (e.g., lung, blood, skin) result in different immunodominant antigenic foci for the immune response [68]. Therefore, a multivalent vaccine may have a greater chance of preventing *S. aureus* infections at multiple anatomical sites than a monovalent vaccine.

Iron acquisition as target antigens

Iron is of fundamental importance to microbial pathogenesis, and *S. aureus* is no exception [83–87]. Prevention of iron uptake by *S. aureus* results in the inhibition of bacterial growth and bacterial death [88, 89]. *S. aureus* has evolved numerous iron uptake mechanisms which facilitate iron acquisition in the host during infection in various anatomical contexts. Many of the proteins involved in iron acquisition have been or are being targeted as vaccine candidates.

The leading vaccine candidate at the current time is based upon the *S. aureus* iron-binding protein (IsdB) [90]. IsdB was initially identified as a promising candidate vaccine antigen by probing *S. aureus* open reading frames in an *Escherichia coli* expression library using serum from patients infected with *S. aureus* or control serum [91]. Proteins identified by the immune serum but not control serum would be recognized as immunogenic after infection with *S. aureus*. Such a screening method is a logical means to identify immunogenic antigens from *S. aureus*, although the resulting protection cannot be inferred from this method. Thus, once IsdB was identified as a robustly immunogenic *S. aureus* antigen across multiple donors' immune serum, the protein was produced and tested for efficacy as a vaccine antigen in a murine model of *S. aureus* i.v. tail vein infection resulting in sepsis [90]. For all six *S. aureus* strains tested, inbred and outbred mice immunized with IsdB plus aluminum hydroxide adjuvant had superior survival post-infection than adjuvant control mice. The protein was found to be highly conserved across *S. aureus* strains. Furthermore, the protein was immunogenic in macaque monkeys. A humanized monoclonal antibody targeting IsdB was found to protect mice in a murine sepsis model and to reduce bacteremia and prevent central venous catheter colonization in a rat model [92].

Three doses (5, 30, or 90 μg) of aluminum adjuvanted IsdB vaccine were compared with saline placebo in a double-blinded, phase I clinical trial [93]. The two higher doses were more immunogenic, and the vaccine was well tolerated. The vaccine was then studied in a very large blinded, randomized, placebo-controlled phase II study of patients undergoing elective cardiothoracic surgery (NCT00518687). Unfortunately, IsdB was the latest *S. aureus* vaccine candidate to fail in clinical trials. The precise reason for the phase II clinical trial being stopped prematurely is not yet completely understood.

IsdA is also a target of immune response during *S. aureus* infection in mice [68]. Another multicomponent vaccine platform targeting iron acquisition in *S. aureus* is being developed by Syntiron in collaboration with Sanofi Pasteur. Other vaccines are in development as well, in trials sponsored by Pfizer and Novartis, both of which are multicomponent vaccines and are entering phase I clinical trials.

An antigen that induces protective Th17-based immunity

Another novel vaccine strategy against *S. aureus* is based on the immunologic cross-reactivity of the candidal

recombinant N-terminus of Als3p (rAls3p-N) vaccine against *S. aureus* cell wall preparations [55, 57]. The precise antigenic targets of *S. aureus* that cross-react to rAls3p-N have not been identified. However, specific immune cross-reactivity between lysostaphin cell wall preparations and rAls3p-N was established [55].

The immunology of this vaccine offers new insights into immunologic mechanisms by which vaccines may be effective at protecting against invasive *S. aureus* infections. The rAls3p-N vaccine induced high antibody concentrations, but these antibodies were not protective when used to passively immunize against *S. aureus* intravenous challenge [55]. Concentrations of anti-rAls3p-N antibodies in individual mice did not correlate well with the risk of death from staphylococcal infection [94]. Furthermore, the vaccine was equally effective in B cell-deficient mice as in wild-type mice, but had no efficacy in T cell-deficient mice [55]. The adoptive transfer of immune $B220^{+}$ B cells did not transfer protection, but the transfer of $CD4^{+}$ T cells did transfer protection. The vaccine was ineffective in IFN-γ- and IL-17A-deficient mice, and in $gp91^{phox-/-}$ mice that are unable to produce superoxide. These mice are therefore used as an animal model for chronic granulomatous disease [94]. Cross-adoptive transfer experiments confirmed that functional phagocytes were operative in vaccine-mediated protection at the downstream effector stage, not the upstream lymphocyte priming stage. Finally, vaccination increased the recruitment and activation of phagocytes at sites of tissue infection in mice, and cytokines produced by vaccine-primed lymphocytes markedly 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 the induction of protective antibodies and by inducing a protective Th1/Th17 response.

Clinical and animal model experience has indicated that hosts deficient in phagocytes, or phagocytic function, are specifically predisposed to *S. aureus* infection [25, 57]. This concept strongly suggests that vaccines can be developed to specifically enhance phagocytic-mediated host defense mechanisms against *S. aureus*. Nevertheless, recent experiences confirm that it is possible to induce and identify protective antibodies even against diseases which are clearly not dependent on antibody-mediated protection. Examples include disseminated candidiasis and invasive aspergillosis [95–97]. Therefore, the available immunopathogenesis data do not preclude the 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 both humoral and cellular responses against the pathogenic organism. Indeed, the latter may be the most likely strategy to result in a strongly protective vaccine against *S. aureus*.

How to target potential patients for a *S. aureus* vaccine development program?

Risk factors for *S. aureus* infections are well described

Although patients with no risk factors frequently developed skin infections caused by *S. aureus* in the community, risk factors associated with a higher risk of developing staphylococcal infections are well characterized. Distinct patient populations develop invasive *S. aureus* infections in health care-associated and in community settings. Health care-associated risk factors for invasive *S. aureus* infections include the presence of indwelling catheters, endotracheal intubation, and foreign bodies (e.g. peritoneal dialysis catheters, prosthetic joints and orthopedic implants, prosthetic heart valves, etc.), stay in an ICU, and surgical procedures/trauma [8, 11, 14]. Patients not in a health care setting who are at a higher risk of developing invasive *S. aureus* infections include patients with chronic defects in cell-mediated immunity (e.g., HIV-infected, those on corticosteroids, etc.); patients with poorly characterized immune dysfunctions associated with metabolic or nutritional disorders (e.g., diabetics, alcoholics, cancer patients, dialysis patients); and patients with congenital phagocytic defects (e.g., chronic granulomatous disease, Chediak–Higashi syndrome, Job's syndrome, Wiskott–Aldrich Syndrome, etc.) [8, 11, 14]. In aggregate, the number of people at risk of an invasive staphylococcal infection numbers in the many millions per year in the USA alone. However, vaccination strategies targeting community versus nosocomial disease would likely differ substantively in development plans.

Vaccines targeting community-onset *S. aureus* infections

More than 90% of community-onset *S. aureus* infections are SSTIs, typically presenting as cutaneous abscesses or cellulitis. Abscesses are treatable with incision and drainage; the need for adjunctive antibacterial therapy in this setting is an open question as available studies to date have been underpowered [98]. However, cellulitis requires antibacterial therapy. Furthermore, the remainder of such infections is more invasive and may lead to community-onset bacteremia, endocarditis, deep tissue abscesses, osteomyelitis, etc. As mentioned, while specific risk factors do identify subjects with higher than normal risk of developing community-onset *S. aureus* infections, everyone is at risk of such infections.

Two general strategies for developing a vaccine against community-onset *S. aureus* infections are apparent (Table 1): first is the large-scale vaccination of the general public to prevent infections on a population basis; second is the focused vaccination on high-risk individuals who have a defined period of extra risk.

Large-scale vaccination of the general public requires a much larger clinical development program, but would result in a much larger market should efficacy be established in a pivotal phase III study. Because so many patients would be vaccinated relative to the small number who would otherwise develop infection in this development strategy, there would be little tolerability for adverse events (even if such events resulted in only mild or temporary changes quality of life rather than in long-term sequelae). Furthermore, it is likely that the market size would be somewhat mitigated by a lower per unit price since the number of infections prevented per vaccinated individual would be relatively small and most of the infections prevented would be non-life-threatening simple abscesses. Finally, such a vaccine would either require prolonged resulting immunity or would require periodic boosting, given the indefinite risk of community-onset infection among the general public.

In contrast, a development strategy for community-onset *S. aureus* infection focused on high-risk individuals would be designed quite differently. Perhaps the most feasible (but not the only) trial design for preventing community-onset *S. aureus* infection in super-high-risk individuals would be to enroll military recruits prior to the initiation of basic training. Multiple publications have described frequent *S. aureus* infections in military personnel [99–104]. A high attack rate in healthy individuals has been found during military basic training [100, 102, 103]. In one study, the attack rate of *S. aureus* infections during just the 8- to 10-week period of basic training for new recruits was 3.5% (29/812). Furthermore, of patients colonized in the nose with *S. aureus* at baseline, the attack rate of *S. aureus* infections during basic training doubled to 7% (17/253). Of patients colonized with MRSA at baseline, the attack rate was much higher (38%). Based on this attack rate in the control arm, if patients known to be colonized with MRSA at baseline were the target population for enrollment in a clinical trial of a vaccine to prevent subsequent infection, only 90 patients per arm would be required to achieve an 80% power to detect a 50% decrease in infection attack rate (α=0.05).

The advantages of studying a vaccine for efficacy in military recruits include a high enrollment rate of eligible study subjects, a relatively high and defined attack rate in the control arm over a short period of time, the enrollment of subjects with healthy immune systems, and the relative ease of data capture given the close monitoring of enrolled subjects that is feasible in such a situation. Such a study would be far smaller and less expensive than would occur in a large-scale!! population-based development program. As well, the tolerability for minor adverse events (such as discomfort at the injection site, brief fever, etc.) would be higher in military recruits than in a program targeting the general population. The trade-off would be the potential loss of efficacy by enrolling subjects already known to be colonized with infection (if, for example, the vaccine's primary benefit was to prevent colonization as an initial step in the pathway eventually leading to infection) and the resulting approval for a narrower indication, which would result in a smaller overall market. However, the smaller market size would be partially mitigated by the likely ability to charge a higher dollar amount per unit dose since the number of infections prevented per vaccine recipient would be substantially higher. Other possible populations at specific risk of focusing a community-onset *S. aureus* vaccine development program could include students or professional athletes, although the attack rate is less well defined in these populations and likely lower than in military recruits. Furthermore, household contacts of index patients [105], prison inmates (at time of entry into prison) [106, 107], and those with a history of prior *S. aureus*

Table 1 Possible clinical development schemes for a *S. aureus* vaccine

Development plan	Community-onset		Health care-associated	
	Population-based	High risk	Nosocomial	Post-discharge
Example population	General public, or those >65 years	Military recruits, athletes, those with previous *S. aureus* infection	AV shunt or graft, previous *S. aureus* infection	All patients at hospital discharge
Target population size	Extremely large	Small	Small	Medium
Attack rate	Very small	Very large	Large	Medium
Severity of prevented infection	Minor	Minor	Severe	Minor
Duration of protection needed	Lifelong	Short (weeks)	Short (weeks)	Unclear
Likely cost per dose	Small	Medium	Large	Medium
Adverse event tolerability	Very low	Medium	Very high	Medium

infection [33–39, 105] all have high subsequent attack rates and would be populations reasonable to target for a clinical trial.

Vaccines targeting health care-associated *S. aureus* infections

In contrast to community-onset infections caused by *S. aureus*, nosocomial infections caused by *S. aureus* are typically life-threatening. Such infections include ventilator-associated pneumonia, catheter-associated bacteremia, and postsurgical wound infections. The prevention of such infections would not just improve quality of life but would also reduce mortality. Furthermore, a recent study found that patients with specific risk factors had rates of *S. aureus* bacteremia in excess of 10% [108]. Hence, targeting patients with these risk factors would enable the achievement of an attack rate feasible for an adequately powered phase III clinical trial. Since the risk of mortality and serious morbidity is greater for hospital-acquired *S. aureus* infections, the quality-adjusted life years saved of administering an anti-*S. aureus* vaccine to prevent hospital-acquired infections would be substantially greater than for preventing community-acquired infections. Hence, even though the number of eligible subjects would be smaller, the cost per dose charged by a sponsor for an indication to prevent hospital-acquired infections would likely be substantially greater than for an indication to prevent community-onset infection.

Another clinical development pathway for an active vaccine is the prevention of reinfection in patients who have experienced a *S. aureus* infection. Lucero et al. [109] conducted a population-based surveillance study of MRSA infections to estimate the impact of a vaccine on preventing such infections. The authors evaluated the potential for a vaccine to prevent MRSA infections in three vaccination strategies, including: (1) all individuals ≥65 years of age, (2) all individuals ≥65 years of age plus persons 15–64 years of age with a history of previous invasive MRSA infection, and (3) all individuals ≥65 years of age plus persons 15–64 years of age at hospital discharge (hospitalization for any indication). Their baseline estimate of disease burden was 38 cases per 100,000, which likely grossly underestimates (by approximately nine- or tenfold) the true incidence of MRSA infections if simple abscesses are included [13, 110]. Thus, the analysis focuses primarily on deep, invasive infections. They estimated that the greatest impact on disease reduction would occur if patients were targeted at hospital discharge (17% reduction in MRSA cases predicted). By comparison, vaccination of all adults >65 years old or all patients with a previous episode of MRSA infection were predicted to result in 12% or 14% reductions in MRSA infections, respectively.

Will compromised hosts respond to a vaccine against *S. aureus*?

A vaccination program focused on the prevention of community-onset infection would predominantly target immune competent individuals. In contrast, a vaccination program focused on preventing health care-associated *S. aureus* infections would by necessity involve vaccinating patients with a variety of comorbidities. In particular, vaccination of acutely ill inpatients at risk of invasive *S. aureus* infections would require the effective vaccination of a variety of types of critically ill patients. The question is raised, are such patients likely to respond to vaccination with a sufficiently potent response to generate protective immunity?

However, numerous clinical studies have confirmed that most patients with even substantial and specific defects in cell-mediated immunity respond to a variety of vaccines. For example, HIV-infected patients and patients with active uncontrolled malignancies, including leukemia, have been shown to generate immune responses to and be protected from infection by a variety of vaccines [111–132]. Therefore, the concept that immunocompromised patients can be protected from invasive infection by immunization has been well validated and extensively documented in both immunogenicity and clinical outcomes studies. Given the established vaccine immunogenicity and clinical efficacy in patients with HIV infection and cancer/chemotherapy-related neutropenia, it is highly likely that patients with poorly characterized immune defects due to acute critical illness will also respond to vaccination.

Summary

Failed efforts to develop a *S. aureus* vaccine to date do not diminish the real potential for effective vaccines to become available in the clinic within the next 10 years. Over the past decade, we have learned critically important lessons regarding the fundamental immunopathogenesis of *S. aureus* infections and regarding the mechanisms of immune protection against infection. Previous *S. aureus* vaccine efforts have focused on capsular polysaccharides as an extrapolation from a half century of successful efforts to develop vaccines against infections caused by typical encapsulated bacterial pathogens. However, the fundamental mechanisms of host defense which protect against typical encapsulated bacterial infections differ from those operative against *S. aureus*. The former are primarily defended against by opsonophagocytic antibodies. In contrast, protection against *S. aureus* infections requires intact phagocytic function and is markedly enhanced by Th1/Th17 adaptive immunity. These facts do not preclude

the possibility of developing a vaccine which is operative by inducing protective antibodies. Rather, they indicate that vaccines targeting *S. aureus* do not *have* to focus on stimulating neutralizing or opsonophagocytic antigens and that cell-mediated focused vaccines can effectively protect against *S. aureus* infections.

Furthermore, these facts indicate that antigenic targets of vaccination do not have to be focused on virulence factors, with the intention of neutralizing such factors and/or inhibiting their function. Any *S. aureus* antigens which induce a potent immune response that effectively targets the organism for destruction by enhancing phagocytic effectors can serve as an effective vaccine antigen, whether the antigen is a capsular component, a cell surface adhesion, a virulence factor, or simply a housekeeping protein which is present at high concentrations and is accessible to immune recognition. More important than the biological role of the microbial antigen is the induction of a potent immune response in as broad a population of targeted patients as possible.

The concept of developing a vaccine based on multivalent antigens has been popularized in recent years. The purported benefit of multivalent antigens has previously been described as targeting multiple virulence factors for a pathogen which is so complex and deploys numerous virulence factors to cause disease. However, perhaps a more relevant promise of multivalent vaccines is the ability to incorporate distinct antigens that (1) induce complementary, non-overlapping immune mechanisms of protection, such as a humoral-inducing antigen and a Th1 or Th17 cell-mediated inducing antigen, and (2) are capable of inducing potent immunity even in diverse human populations which intrinsically respond to myriad different *S. aureus* antigens with great variability. In contrast, a single antigen will only be effective in a smaller subset of patients who respond particularly well to that specific antigen and may not be capable of inducing potent protection by both humoral- and cell-mediated immunity.

A wide variety of clinical development programs should be feasible for a *S. aureus* vaccine targeting community-onset or health care-associated infections. These varied possible programs will facilitate clinical development by providing numerous options to companies of varying sizes and financial resources and also create multiple niches which could accommodate multiple vaccines in the market place. Given all that has been learned about *S. aureus* immunopathogenesis and vaccinology in the last decade, there is reason for considerable optimism that one or more *S. aureus* vaccines will become available for clinical use in the coming decade.

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