Challenges for a Universal *Staphylococcus aureus* Vaccine

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This review considers the reasons why the staphylococcal vaccine trials may have failed, based on new information about protective immunity against *Staphylococcus aureus*. The clinical trials and future vaccine candidate antigens are reviewed. Challenges facing the development of a universal *S. aureus* vaccine are also considered. The lack of a biomarker that is able to predict protection is a major stumbling block in the development of a staphylococcal vaccine. The major new information involves the role of cell-mediated immunity, specifically T-helper 17 cells and interleukin 17, as well as the lack of protection afforded by specific antibodies. This has major implications for future vaccine development and planning of clinical trials.

Mortality, morbidity, and cost from invasive *Staphylococcus aureus* infections remain disturbingly high despite the introduction of several new antibiotics to treat methicillin-resistant *S. aureus* infections [1–5]. *Staphylococcus aureus* infections are now the most common cause of hospitalization for surgical drainage of pus in children, the most common cause of bacteremia in persons aged >65 years, and the most serious cause of prosthetic device and intravascular line infection. To address these problems, investigators at universities, institutes, biotechnology companies, and large pharmaceutical companies have tried to develop an effective vaccine [6–12]. Despite much effort, no clinical trials have succeeded to date.

Bacterial vaccines have successfully reduced the number of serious infections caused by *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and toxigenic bacteria causing diphtheria, pertussis, and tetanus [13]. What has made *S. aureus* such a difficult vaccine candidate? First and foremost, the pathogenic mechanism and immunity against the pathogens listed above were clearly understood before vaccine development, and they revolved around surface antigens and toxins that were virulence factors that could be neutralized by antibodies. Protective immunity against *S. aureus* is not completely understood. Animal models, especially murine models, have not predicted success in humans as they did for the other successful vaccines. Second, the situation for *S. aureus* is much more complex, as this bacterium has multiple virulence factors, including hemolysins, toxins, and superantigens [14]. Third, *S. aureus*-infected patients present with a very broad range of diseases, which means that vaccine development must focus on preventing a wide spectrum of disease presentations [14]. Fourth, *S. aureus* has a much more extensive array of pathogenicity factors that neutralize the host immune responses than these other bacterial pathogens, probably because it lives with us as normal flora [14].

PROTECTIVE IMMUNITY TO *S. AUREUS*

In each of the vaccine clinical trials completed to date [Table 1], target antigens were designed to elicit antibody responses. All of the trials have ended in failure. We may now evaluate these vaccine failures in light of our expanding knowledge of native immunity to *S. aureus*. Observations in human patients as well as murine models are shedding light on immune mechanisms necessary for protection.
Although healthy persons naturally have high titers to *S. aureus*, patients with defects in humoral immunity are not particularly prone to *S. aureus* infections [24, 25]. For example, patients with hereditary (X-linked) agammaglobulinemia rarely have clinically important *S. aureus* infections, although *Streptococcus pneumoniae* and *Pseudomonas* species are clinically important in these patients [26, 27], often leading to death. The lack of antibody against *S. aureus* must be compensated for by other immune mechanisms. This apparent lack of antibody contribution to *S. aureus* disease prevention is somewhat surprising because both antibody and complement enhance the bactericidal effect of neutrophils. Patients with neutrophil disorders, such as chronic granulomatous disease and Job’s syndrome, suffer from an increased incidence of *S. aureus* infections [24, 25]. This apparent paradox may be resolved by recent information on the importance of T-helper 17 (Th17) cells and interleukin 17 (IL-17) for protection against *S. aureus* infections [28, 33].

### Table 1. Summary of Clinical Trials Based on Increased Opsonophagocytic Activity

<table>
<thead>
<tr>
<th>Company</th>
<th>Antigen</th>
<th>Patients</th>
<th>Outcome</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>Active immunization</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Nabi</td>
<td>Types 5 and 8 CPS conjugated to pseudomonal exoprotein A</td>
<td>Hemodialysis</td>
<td>Failed phase 3</td>
<td>Shinefield et al [15]</td>
</tr>
<tr>
<td>GlaxoSmithKline</td>
<td>Nabi vaccine + wall teichoic acid + PVL (rLukS-PV/AT) + Hla</td>
<td>Immunogenicity</td>
<td>Phase 1</td>
<td>ClinicalTrials.gov NCT01160172</td>
</tr>
<tr>
<td>Pfizer</td>
<td>Types 5 and 8 CPS + ClfA</td>
<td>Volunteers</td>
<td>Phase 1 dose evaluation</td>
<td>ClinicalTrials.gov NCT01018641</td>
</tr>
<tr>
<td>Merck</td>
<td>IsdB (V710)</td>
<td>Cardiotoracic surgery; prevention of bacteremia and wound infection</td>
<td>Futility in phase 2/3 (August 2011)</td>
<td>ClinicalTrials.gov NCT00618887</td>
</tr>
<tr>
<td>Novadigm</td>
<td>rAl3p-N (C. albicans surface protein that cross-reacts with <em>S. aureus</em>)</td>
<td>Volunteers</td>
<td>Phase 1 dose evaluation</td>
<td>ClinicalTrials.gov NCT01447407</td>
</tr>
<tr>
<td>VRI</td>
<td>Eap, GST-Can, His-Clf</td>
<td></td>
<td>Phase 1</td>
<td><a href="http://www.vri.org.uk/phaseITrial.html">http://www.vri.org.uk/phaseITrial.html</a></td>
</tr>
<tr>
<td><strong>Passive immunization</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Integrated Bio-Therapeutic</td>
<td>SEB (STEBVax)</td>
<td>Toxic shock syndrome</td>
<td>Phase 1 suspended</td>
<td>ClinicalTrials.gov NCT00974935</td>
</tr>
<tr>
<td>Inhibitex</td>
<td>ClfA, SdrG (Veronate)</td>
<td>Neonates</td>
<td>Failed in 2 trials</td>
<td>DeJonge et al [16]; Patti [17]</td>
</tr>
<tr>
<td>Inhibitex</td>
<td>Anti-ClfA mAb tofibazumab (Aurexis)</td>
<td>Adults, bacteremia</td>
<td>Phase 2 trial: decreased relapse and complications, no lower mortality; hypersensitivity reaction developed in 1 of 30</td>
<td>Weems et al [18]</td>
</tr>
<tr>
<td>Nabi</td>
<td>Polyclonal anti-CPS 5 and 8 (Altastaph)</td>
<td>Neonates or young children</td>
<td>Failed</td>
<td>Benjamin et al [19]; Rupp et al [20]</td>
</tr>
<tr>
<td>BioSynexus</td>
<td>Anti-LTA (pagibaximab)</td>
<td>Low-birth-weight neonates for prevention of bacteremia</td>
<td>Failed phase 3 to reduce mortality</td>
<td>Weisman [21]; ClinicalTrials.gov NCT00646399</td>
</tr>
<tr>
<td>NeuTec Pharma</td>
<td>ATP-binding cassette transporter (Aurograb)</td>
<td>Combined with vancomycin</td>
<td>Failed phase 3</td>
<td>Burnie et al [22]; Garmory and Titball [23]; ClinicalTrials.gov NCT00217841</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, adenosine triphosphate; *C. albicans*, *Candida albicans*; ClfA, fibrinogen adhesin; CPS, capsular polysaccharide; Eap, extracellular adherence protein; GST-Can, collagen binding protein; His-Clf, clumping factor; Hla, α-toxin (α-hemolysin); IsdB, iron surface determinant B; LTA, lipoteichoic acid; mAb, monoclonal antibody; PVL, Panton-Valentine leukocidin; *S. aureus*, *Staphylococcus aureus*; and SdrG, *S. epidermidis* fibrin-binding protein; SEB, *Staphylococcal Enterotoxin B*. 

1180 • CID 2012:54 (15 April) • CLINICAL PRACTICE
a lack of Th17 cell development [29–32]. Th17 cells produce IL-17, which is crucial for neutrophil recruitment and activation [33]. Of interest, mice that are IL-17A/-F-/- develop spontaneous S. aureus skin abscesses [34]. Humans with IL-17 defects are also prone to S. aureus skin infections [35]. Interleukin 17 is produced by Th17 cells as well as innate immune cells, such as γδ T cells, and natural killer T cells [36] and is one of the cytokines important for calling in and activating neutrophils for killing S. aureus [36, 37]. This ties together cell-mediated immunity (CMI) and neutrophil effector activities, wherein antibodies may play a supportive role for opsonization and/or neutralization of virulence factors.

Although patients with neutrophil disorders develop deep-seated S. aureus and Candida albicans infections [25], patients with CMI defects develop skin and mucocutaneous infections with these organisms [35–41]. Why should patients harboring these neutrophil-cleared pathogens present with predominantly mucocutaneous disease? This most likely relates to the effects of IL-17 on activation of keratinocytes and mucosal cells at these protective barriers [42, 43]. Interleukin 17 is necessary to stimulate keratinocytes and epithelial cells to produce antimicrobial peptides and recruit neutrophils. This is consistent with the observations that Th17 cells are critical for protection of mice from naturally occurring skin and lung infections [44]. When one considers community-associated methicillin-resistant S. aureus infections, one is struck by the number of cases of skin infections [45, 46] and the increased severity of lung infections with necrotizing pneumonia [45]. Perhaps, although antibodies may limit some of the invasive effects of S. aureus, the skin and mucosal barriers, including the innate mediators in these barriers, are keys to preventing initial infections at these sites.

As might be anticipated from data implicating Th17 cells, patients with other defects in CMI (eg, high prednisone therapy, human immunodeficiency virus [HIV]/AIDS, and defective interferon γ production) have an increased incidence of S. aureus infections [47–51]. Recurrent community-acquired S. aureus represented 6.8% skin and soft tissue infections in HIV-infected patients with an incidence rate of 12.3 infections per 1000 person-years [47]. Two other studies showed a 6-fold increase of skin and soft-tissue infections in HIV-positive patients compared with HIV-negative patients in ambulatory care settings [49, 51]. These studies underscore the importance of CMI in protecting the host from S. aureus infections.

Neither immunoglobulin G nor B cells are necessary for recovery from S. aureus infection in a mouse model of septic arthritis [52, 53]. However, mice with specific immune defects in Th17 cells and/or IL-17 support were more susceptible to S. aureus infections, which supports human data implicating this arm of the immune system. Additionally, Th17 cells plus neutrophils were found necessary for protection against S. aureus challenge in murine models [43, 54, 55]. Interleukin 17 receptor and γδ T-cell–deficient mice were shown to have increased abscess size and numbers of colony-forming units (CFUs) per lesion after S. aureus challenge compared with wild-type mice. Treatment of the γδ T-cell–deficient mice with IL-17, but not interleukin 21 or interleukin 22 (IL-22), reduced the abscess size and lesion CFU numbers to levels observed in the wild-type mice [43]. Studies using B–cell–defective BALB/c mice do not indicate an increased mortality of the mice when challenged intravenously with S. aureus [55], but mice suffer markedly increased lethality when challenged with S. pneumoniae or H. influenzae. Further support for Th17/IL-17 being critical for protection from S. aureus infections comes from investigations of Als3p, a cross-protective antigen from C. albicans [54, 55]. Protection against S. aureus challenge after rAls3p-N (a recombinant subunit of Als3p) immunization was found to be mediated by T cells, specifically Th17 and T-helper 1 cells [54, 55]. This protection was lost in mice that lacked neutrophils producing reactive oxygen species (ie, gp91phox-/- mice) [55]. Although immunized B–cell–deficient mice were protected, T-cell–deficient mice died after S. aureus challenge. Adoptive transfer of Als3p immune CD4+ T cells provided protection, but B cells and antibodies were not protective. In other studies, IL-17A–deficient C57BL/6 mice immunized with ClfA, a fibrinogen-binding surface protein involved in staphylococcal adhesion to host tissues, were not protected, whereas their parent strain was protected [56]. Therefore, both human clinical observations and animal models strongly support the concept that CMI and, specifically, Th17/IL-17 play a central role in protection against S. aureus infections.

Further support for the involvement of Th17 cells in S. aureus immunity comes from studies of bacterial pneumonia in mice [57]. Influenza A was found to inhibit Th17-mediated host defense in mice via induction of type 1 interferons. This led to impaired bacterial clearance of S. aureus and resulted in increased mortality. Treating mice with exogenous interleukin 23 (IL-23) stimulated production of IL-17 and IL-22, which markedly improved clearance of S. aureus and reduced mortality. Of interest, the highly virulent community-associated methicillin-resistant S. aureus strains can induce type 1 interferons within the lungs of mice [58], thereby inhibiting the Th17 cell pathway. These observations are consistent with the increased frequency and severity of S. aureus as a complication of influenza [59].

Of note, a recent report found that heat-killed S. aureus provided protection via staphylococcal lipoproteins that stimulated IL-17 through Toll-like receptor (TLR) 2–MyD88 activation, but T and B cells were not required for vaccination-mediated protective effect [60]. This surprising finding in a murine peritoneal infection model is apparently contrary.
to clinical and experimental data reported elsewhere, in which T cells seem to be critical for protection. Interleukin 17 can be produced by innate cells of the immune system, preempting the need for T and B cells or Th17 immunity [37], which may help to explain these results. Additionally, the data of Schmaler et al [60] may be consistent with the data of Mele et al [61], wherein the authors demonstrated that heat-killed S. aureus immunization produced immunomodulatory effects from interleukin 10 released via the TLR2/6 and MyD88 pathway. Induction of this pathway reduced murine susceptibility to the cytokine storm caused by superantigens, thereby reducing mortality. Naturally, this immunomodulatory effect complicates conclusions when examining the vaccine immunological effects of heat-killed bacteria. The report by Schmaler et al also underscores the differences in immune response to whole, killed bacteria versus acellular protein antigens. This difference may become key when considering the design of future vaccines. For example, a key role of Th17 cell responses was found in the protective efficacy afforded by the acellular pertussis vaccine [62], whereas the whole cell vaccine provided protection via antibodies to surface components.

Defining the specific arm of the human immune system that affords protection from S. aureus infection is further complicated by the protection afforded by the innate immune system. Toll-like receptor 2/6 and MyD88 pathways are activated by staphylococcal surface components [61, 63]. Moreover, S. aureus α-toxin activates the immune system via a TLR2-independent mechanism whereby NOD2 signaling results in protection against S. aureus murine skin infections [64, 65]. Another complicating factor is that S. aureus possesses multiple virulence factors that thwart the immune system. This includes toxins that lyse phagocytes, capsules that prevent efficient opsonization, adhesins to host tissues that allow uptake into nonprofessional phagocytes thereby providing a site hidden from the immune system, and anti-immune system factors that target antibodies, complement, selectins, ICAM-1, and innate immune system receptors [66–68]. Although all of these have implications for vaccine development, perhaps the one least studied for its impact on human vaccines has been protein A, which inhibits the opsonic activity of antibodies [10].

Finally, there may be genetic variability among humans that predisposes them to S. aureus infections [61]. As an example of this, it has been observed that 6%–8% of the white population carries single-nucleotide polymorphisms in the gene for the IL-23 receptor, potentially affecting CD4+ cell conversion to Th17 cells and thus responses to S. aureus [69]. Single-nucleotide polymorphisms, which may impact vaccine efficacy, have not been exhaustively studied.

Although a growing body of evidence implicates a critical role of Th17 cells for protection from naturally occurring skin and lung infections, Th17/IL-17 seems to be less critical for other sites [30]. In all likelihood, multiple arms of the immune system will be important for vaccine-mediated S. aureus immunity. Biomarkers based on a clear understanding of the protective immune response have been critical to development of bacterial vaccines (e.g., the presence of anticapsular antibodies in humans strongly correlated with protection from invasive S. pneumoniae infections). This allowed epidemiological studies to identify at-risk populations wherein a vaccine could be tested. To decrease mortality from S. aureus pneumonia, a robust Th17 response plus anti-Panton-Valentine leukocidin and Hla antibodies might be a combination of biomarkers to predict success.
Staphylococcus aureus provides a number of unique challenges compared with other bacteria for which successful vaccines have been developed [Table 2]. Because humans live in frequent contact with S. aureus, all normal persons have S. aureus antibodies (of which a portion are received as maternal antibodies, with the rest developing beginning shortly after birth [70, 71]), their innate immune system is activated, and S. aureus is armed with multiple factors to disarm host immune responses. Our focus on antibodies, as well as our limited knowledge of the definitive immune protective responses, has resulted in the development of vaccine candidates that cannot provide more protection than is already available in most persons. Additionally, we may have overlooked the potential for stimulating the highly evolved native host defenses at skin and mucosal surfaces. Examination of one vaccine candidate, iron surface determinant B (IsdB), may be instructive.

Antibody levels to IsdB correlate with protection in preclinical models, enhancing opsonic activity of H160 cells and reducing biofilms on catheters [72–77], and IsdB-specific murine and human monoclonal antibodies mediated opsonic activity optimally at 100–200 µg/mL [72, 74]. Human IsdB-specific monoclonal CS-D7 protects against a very high challenge dose (approximately 2 × 10⁹ CFUs) at 17–20 mg/kg in a murine lethal sepsis model and at 12–13 mg/kg in a rat indwelling-catheter model. Based on supportive preclinical data, IsdB has been studied for the prevention of S. aureus infections in patients undergoing elective cardiovascular surgery (V710, protocol 003; www.clinicaltrials.gov: NCT00518687). For this efficacy trial, patients were immunized with a single injection of 60 µg of lyophilized unadjuvanted 0657nI (truncated IsdB) 2 weeks before surgery. In prior phase 1 testing, immunization induced an anamnestic antibody response to the vaccine, and by day 10 after immunization, 81% of patients immunized had antibody titers of twice the prevaccination level (www.clinicaltrials.gov: NCT01324440) [78, 79]. Antibodies induced by the vaccine had opsonic activity. However, the V710 protocol 003 trial was terminated after a regularly scheduled interim data analysis due to lack of efficacy (Merck, Intercell press release, http://www.merck.com/newsroom/news-release-archive/research-and-development/2011_0608.html, 8 June 2011). Thus, although ample preclinical data supported the potential efficacy of antibodies specific to IsdB, the human efficacy trial did not meet the study goals. Although early work indicated polyclonal antibody levels correlated with protection in IsdB-immunized mice, the type of immunity generated was not defined [75].

More recently, Th17/IL-17 immunity has been shown to be determinative in IsdB-immunized mice challenged with S. aureus. Joshi et al [80] reported that IsdB-immune T cells were critical for protection of mice with severe combined immunodeficiency (SCID) challenged with a lethal dose of S. aureus via the tail vein, whereas neither active immunization nor passive transfer of monoclonal antibodies against IsdB provided protection to the mice with SCID. This implicated T cells as being important. Adoptive transfer of IsdB immune CD3/4⁺ cells protected the mice with SCID, whereas neither CD8⁺ T cells, nor B cells, nor plasmacytes were protective. Immunization with IsdB was shown to be critical for activating the transferred T cells, because T cells from bovine serum albumin-immunized mice were not protective. To dissect the immune response further, it was shown using intracellular staining that IsdB-immune CD4⁺ cells stimulated with IsdB produced IL-17 and that IL-17, but not IL-22 or interferon γ, was necessary for IsdB-generated protective immunity. Finally, Th17 immunity was specific for IsdB because challenging mice replenished with IsdB immune T cells with an isdB/harA-deleted strain (HarA is an IsdB homolog) did not result in protection. Thus, IsdB vaccine-mediated murine protective immunity to S. aureus infections is specific and dependent on Th17/IL-17. Although the human Th17 cell response to V710 is not currently known, the murine data may indicate that vaccines designed to prevent S. aureus infections may need to be administered with a T-cell stimulating adjuvant. The Merck V710 trial required that patients have surgery within 14–60 days after vaccination, making loss of Th17 memory unlikely. A robust level of vaccine-induced antibodies may be important, but insufficient, for inducing protective efficacy.

An unanswered question concerning the Th17 cell response is duration of Th17 cell memory. In murine experiments, Th17 immunity lasted about 3 months in mice after intranasal immunization [81]. If Th17 immunity is critical for S. aureus vaccines, then defining the duration of immunity in humans will be important in vaccine development. Results of the first Nabi capsule conjugate vaccine trial seemed to indicate efficacy from week 3 to week 40, but bacteremia actually increased during the later stage of the trial with a loss of net protective effect despite very high, though falling, levels of antibodies [15]. Although we do not know whether the antigens used in the Nabi capsular vaccine trial induced a Th17/IL-17 response, it is tempting to speculate that a decrease in efficacy might be the result of insufficient IL-17 production in this setting. In addition, a relatively short duration of Th17 immunity might explain the recurrent S. aureus skin infections seen in some patients [82, 83]. To address the issue of fading immunity, adjuvants that drive a Th17 cell response might produce better short- and long-term protection [81]. Clearly, the acellular pertussis vaccine, which stimulates a Th17 cell response, does produce
longer-term immunity, but recent reports of a record number of cases from several locations in the United States raise questions about the duration of the acellular vaccine [84]. Perhaps longer-term immunity can be obtained in humans to Staphylococcus aureus antigens that induce a strong Th17 cell response. Alternatively, booster doses may need to be given based on the duration of Th17 immunity.

CONCLUSIONS

Attempts to produce an effective Staphylococcus aureus vaccine based on the production of antibodies may have failed for several reasons. The correct antigen or combination of antigens may not have been selected. It is possible that the antibodies were oposic but did not produce bactericidal activity in neutrophils or that inhibitory antibodies were present [85]. Clearly, animal models, although informative, are not predictive of efficacy in humans. We should consider the natural and acquired immune defects wherein Staphylococcus aureus infections are more commonly related to reduced CMI and less related to antibody production.

The failure to prevent infection or reduce mortality (Table 1) and the unique characteristics of Staphylococcus aureus (Table 2) have direct implications for clinical trial design. First, without a reliable biomarker, it is difficult to design a clinical trial or to provide a cogent reason why the trials failed, in contrast to the pneumococcal vaccine, in which opsonic antibody to capsular polysaccharide was a clear biomarker in animal and human infections. Second, which of the many diseases Staphylococcus aureus causes does one want to prevent? If a clinical trial were aimed at a narrow-spectrum disease, such as Staphylococcus aureus pneumonia in intensive care unit patients, then one could aim to develop neutralizing antibodies for Panton-Valentine leukocidin and α-toxin. There are epidemiological correlations between these toxins and severity of human hemorrhagic pneumonias. Third, because prevention of disease may not be possible with an organism that routinely colonizes the host, the design of clinical trials should be to aim at the secondary end points, such as disease severity (e.g., fever, white blood cell count, shock, respiratory failure) and reduced length of stay, rather than disease prevention, which may not be possible given the ability of Staphylococcus aureus to live with the host. Therefore, the design of future clinical trials should probably aim at narrower indications and rely on human epidemiological data rather than animal models.

Recent studies on the Th17/IL-17 axis may provide avenues for development of a broadly effective Staphylococcus aureus vaccine that prevents infections. However, this may not be possible, and we may need to redirect our thinking toward developing a vaccine that causes infections to be less severe and invasive, thereby reducing morbidity and mortality. In any event, the need for an effective Staphylococcus aureus vaccine is increasing in view of broad antibiotic resistance and apparent increasing virulence of the community strains.

Note

Potential conflicts of interest. Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


