Vaccination and passive immunisation against *Staphylococcus aureus*®

Adam C. Schaffer, Jean C. Lee *

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

**A B S T R A C T**

*Staphylococcus aureus*, an important bacterial pathogen in the hospital and the community, has become increasingly resistant to multiple antibiotics. Non-antimicrobial approaches to controlling *S. aureus* are clearly needed. Because many individuals who are susceptible to staphylococcal infections are not competent to mount an effective immune response, passive as well as active immunisation strategies have been explored. A capsular polysaccharide-based vaccine (StaphVAX®) showed promise in an initial phase III trial in haemodialysis patients, but was found to be ineffective in a confirmatory trial. Likewise, a human immunoglobulin G (IgG) preparation known as INH-A21 (Veronate®) with elevated levels of antibodies to the staphylococcal surface adhesins ClfA and SdrG made it into phase III testing, where it failed to show a clinical benefit in neonates. A number of novel antigens are in pre-clinical trials, including cell-wall-anchored adhesins, surface polysaccharides and exotoxoids. Given the multiple and sometimes redundant virulence factors of *S. aureus* that enable it to be such a crafty pathogen, if a vaccine is to prove effective it will of necessity be multicomponent, incorporating a number of surface proteins, toxoids and surface polysaccharides.

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1. Introduction

*Staphylococcus aureus* is an important bacterial pathogen that causes skin and soft-tissue infections as well as life-threatening bacteraemias with metastatic complications, such as pneumonia, endocarditis, septic arthritis and osteomyelitis. The staphylococcus is a well-established pathogen in the hospital, where it causes infections in immunocompromised hosts, surgical patients and in those with indwelling medical devices. Meticillin-resistant *S. aureus* (MRSA) strains are responsible for nosocomial and community-acquired staphylococcal infections, and many of these isolates are multidrug resistant. Because *S. aureus* cannot always be controlled by commonly used antibiotics and because MRSA isolates are becoming increasingly prevalent in the community, additional control strategies are greatly needed. An *S. aureus* vaccine offers a mechanism to boost the immune system so that effector molecules elicited by the host will contain and eradicate the infecting microbe.

2. Is an effective *S. aureus* vaccine feasible?

Successful vaccine design relies on an understanding of how the pathogen relates to the host. The protean clinical manifestations of staphylococcal infections still leave many gaps in our understanding of the interactions between the host and this versatile microbial pathogen. Importantly, little evidence supports the premise that immunity to *S. aureus* infection exists, at least for the non-immunised host. With the recent increase in community-associated staphylococcal infections, *S. aureus* is now commonly isolated from individuals with no predisposing risk factors. Recovery from an *S. aureus* infection does not appear to confer immunity against subsequent infections. *Staphylococcus aureus* produces a large array of molecules with redundant functions, such that if one is eliminated (or targeted by a vaccine), other staphylococcal products may compensate for that loss of function. Other challenges to vaccine development include the diverse strategies that *S. aureus* has developed to avoid human innate immunity as well as its ability to persist in biofilms and as small-colony variants. Animal models of *S. aureus* infection are imperfect, requiring large inocula to establish infection. Unlike humans, laboratory rodents do not have serum...
3. Target populations

A crucial question in vaccine development is the target population for an *S. aureus* vaccine. There are certain groups (Table 1) that are likely candidates for active immunisation against staphylococcal disease. Passive immunoprophylaxis against staphylococcal infections is indicated for persons who are unable to respond to active immunisation because they are immunocompromised. Passive immunotherapy would also be appropriate for individuals who are at immediate risk of infection and for whom time constraints prohibit an active immunisation approach.

4. Active immunisation approaches in clinical trials (Table 2)

4.1. StaphVAX®

Most *S. aureus* strains are encapsulated, and strains producing either capsular polysaccharide serotype 5 (CP5) or serotype 8 (CP8) (Fig. 1) are the most prevalent among clinical isolates. Capsular antigens are obvious targets for vaccine development, since capsule-based vaccines directed against other encapsulated bacterial pathogens have shown high success rates. Fattom et al. [1] conjugated CP5 and CP8 to recombinant *Pseudomonas aeruginosa* exoprotein A. The conjugate vaccines were highly immunogenic, and antibodies elicited by immunisation opsonised encapsulated *S. aureus* for phagocytosis. Passive immunisation with antibodies to CP5 was protective in a mouse model of *S. aureus* lethality.

### Table 1

<table>
<thead>
<tr>
<th>Active immunisation</th>
<th>Passive immunisation</th>
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<tbody>
<tr>
<td>Haemodialysis patients</td>
<td>Patients undergoing emergency surgery</td>
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<tr>
<td>Residents of nursing homes and other long-term care facilities</td>
<td>Patients implanted with intravascular or prosthetic devices</td>
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<tr>
<td>Men who have sex with men</td>
<td>Trauma victims</td>
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<tr>
<td>Military personnel</td>
<td>Immunocompromised individuals</td>
</tr>
<tr>
<td>Prisoners</td>
<td>Low birthweight neonates</td>
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<tr>
<td>Patients undergoing elective surgery</td>
<td>Patients in intensive care units</td>
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<tr>
<td>Individuals with diabetes</td>
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<tr>
<td>Patients with HIV</td>
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<td>Intravenous drug users</td>
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<td>Healthcare providers</td>
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<tr>
<td>Athletes</td>
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<tr>
<td>School children</td>
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</table>

HIV, human immunodeficiency virus.

Antibodies to *S. aureus* antigens and this could affect infection outcome and vaccine efficacy.

Fig. 1. (Top) Transmission electron micrographs of stationary phase *Staphylococcus aureus* cells. Prior to fixation, the bacteria were incubated with capsular polysaccharide serotype 5 (CP5)-specific antibodies to stabilise and visualise the capsule. (Left) CP5-producing strain Reynolds; (right) acapsular *S. aureus* mutant. (Bottom) Structural composition of *S. aureus* CP5 and CP8. ManNAcA, 2-acetamido-2-deoxy-mannuronic acid; FucNAc, 2-acetamido-2,6-dideoxy-galactose. Adapted from O’Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev 2004;17:218–34 with permission. Copyright 2004 American Society for Microbiology.
Table 2

<table>
<thead>
<tr>
<th>Product</th>
<th>Corporate sponsor</th>
<th>Composition</th>
<th>Status</th>
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<tbody>
<tr>
<td>Active immunisation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>StaphVAX</td>
<td>Nabi</td>
<td>CP5 and CP8</td>
<td>Phase III failed</td>
</tr>
<tr>
<td>V710 (0657nl)</td>
<td>Merck</td>
<td>IsdB</td>
<td>Phase II in progress</td>
</tr>
<tr>
<td>Passive immunisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INH-A21 (Veronate)</td>
<td>Inhibitex</td>
<td>CIFA (selected IVIg)</td>
<td>Phase III failed</td>
</tr>
<tr>
<td>Tefibazumab (Aurexis)</td>
<td>Inhibitex</td>
<td>CIFA (mAb)</td>
<td>Phase II completed</td>
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<tr>
<td>AltaStaph</td>
<td>Nabi</td>
<td>CP5 and CP8</td>
<td>Phase II completed</td>
</tr>
<tr>
<td>Aurograb</td>
<td>NeuTec</td>
<td>ABC transporter</td>
<td>Phase II completed</td>
</tr>
<tr>
<td>Pagibaximab (BSYX-A110)</td>
<td>Biosynexus</td>
<td>Lipoteichoic acid</td>
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CP, capsular polysaccharide; IVIg, intravenous immune globulin; mAb, monoclonal antibody; ABC, ATP-binding cassette.

and disseminated infection and in a rat model of catheter-induced staphylococcal endocarditis.

Nabi Biopharmaceuticals combined the CP5 and CP8 conjugate vaccines into a bivalent vaccine called StaphVAX for immunisation of humans at risk for *S. aureus* infection. The first phase III clinical trial tested whether the vaccine would prevent *S. aureus* bacteraemia in endstage renal dialysis patients during the period from Week 3 to Week 54 after immunisation [2]. At the conclusion of the trial (Week 54) the cumulative reduction in episodes of *S. aureus* bacteraemia was only 26% (*P* = 0.23) (Fig. 2). However, if earlier time intervals were analysed (Weeks 3–40), StaphVAX was reported to reduce the incidence of *S. aureus* bacteraemia by 57% (*P* = 0.02). During this period, *S. aureus* bacteraemia occurred among 11 of 892 patients who received the vaccine compared with 26 of 906 control patients.

A confirmatory phase III clinical trial involved 3600 haemodialysis patients who were evaluated for bacteraemia from 3–35 weeks after receipt of StaphVAX. Following a booster dose, the patients were followed for an additional 6 months. Results from the second trial, announced in November 2005 (not yet published), revealed that StaphVAX offered no significant protection against bacteraemia over the placebo control. Although Nabi attributed the clinical failure of the vaccine to the immunocompromised status of the patients in the trial and a manufacturing problem in vaccine production, the data suggest that a conjugate vaccine that targets the *S. aureus* CPs alone is insufficient to protect against staphylococcal bacteraemia.

Why is a CP conjugate vaccine protective against pathogens such as *Haemophilus influenzae* type b and *Streptococcus pneumoniae*, but not *S. aureus*? Antibodies to CP5 and CP8 have been shown to opsonise *S. aureus* for phagocytic killing by human neutrophils. However, *S. aureus* shows only a modest reduction in virulence in animal models of abscess formation, arthritis, wound infection and bacteraemia in the absence of CP expression [3]. Moreover, capsule-negative mutants are more virulent than the parental isolates in a catheter-induced endocarditis infection model. Only ca. 75–80% of *S. aureus* clinical isolates are encapsulated by CP5 or CP8; the remaining strains produce no CP due to mutations in the cap5(8) locus or in genes that regulate CP expression [4]. Importantly, serotype 5 and 8 *S. aureus* elaborate CPs in vitro only during the stationary growth phase; thus, actively replicating staphylococci are acapsular [3,5].

4.2. Vaccine V710 (IsdB)

Etz et al. [6] identified IsdB as a candidate vaccine antigen by probing an *S. aureus* peptide expression library displayed on *Escherichia coli* with sera from patients with high antibody titres.
and opsonic activity. The cell-wall-anchored Idb protein, conserved among diverse clinical isolates of S. aureus, is expressed only under conditions of limiting iron. It binds to haemoglobin and this interaction plays a role in the acquisition of heme iron by S. aureus. Compared with sham-immunised animals, mice immunised with recombinant Idb showed improved survival following intravenous challenge with five of six clinical strains of S. aureus. Protection was not observed when the immunised animals were challenged with an Idb mutant strain, demonstrating the specificity of the protective response [7].

Merck’s V710 vaccine (previously designated 0657nl) completed phase I testing and is currently being tested in several phase II clinical trials for the prevention of S. aureus infection in patients undergoing cardiothoracic surgery as well as in haemodialysis patients.

5. Passive immunisation approaches in clinical trials (Table 2)

5.1. Staphylococcus aureus clumping factor A (ClfA)-based products

ClfA is a cell-wall-anchored adhesin that mediates S. aureus binding to fibrinogen and promotes the attachment of S. aureus to biomaterial surfaces, blood clots, platelets and damaged endothelial surfaces. Pre-clinical studies by scientists at Inhibitex revealed that mice immunised with ClfA showed reductions in arthritis and lethality induced by S. aureus, but protection was strain-dependent [8]. Therapy with ClfA antibodies and vancomycin resulted in better bacterial clearance from the blood of rabbits with catheter-induced S. aureus endocarditis than vancomycin treatment alone [9]. However, the bacterial burdens in the tissues of the infected animals were not significantly reduced.

A related Inhibitex product, INH-A21 (Veronate®), is a pooled human immunoglobulin (Ig) preparation from donors selected for high antibody titres against staphylococcal adhesins that bind fibrinogen and fibrin (S. aureus ClfA and Staphylococcus epidermidis SdrG). This product protected neonatal rats from S. epidermidis infection compared with normal IgG. Pre-treatment of rabbits with INH-A21 significantly decreased the infection rates both of S. aureus and S. epidermidis in a catheter-induced endocarditis infection model [10].

A phase II trial conducted from 2002 to 2003 studied escalating doses (250, 500 or 750 mg/kg) of INH-A21 in premature neonates [11]. The rate of S. aureus infection in the highest INH-A21 dose group (2.5%) was lower (P = 0.14) than the rate of S. aureus infections in the placebo group (7.0%). Infants who received up to four doses of INH-A21 (750 mg/kg) also showed trends towards fewer episodes of candidaemia and lower mortality than the placebo-treated cohort.

These findings prompted a phase III trial of INH-A21 in 1983 neonates who received either placebo or 750 mg/kg INH-A21 [12]. The primary outcome measure was the rate of late-onset S. aureus sepsis and there was no significant difference between the 6% rate in the INH-A21 group and the 5% rate in the placebo group (P = 0.34) (Table 3). Similarly, there were no differences between the groups in mortality or in the rates of late-onset sepsis caused by coagulase-negative staphylococci (CoNS) or Candida spp. These results were particularly disappointing since, in effect, the INH-A21 product likely contained antibodies to many other staphylococcal antigens and could loosely be considered a ‘multicomponent’ passive immunotherapy. However, the INH-A21 product was not elicited by immunisation but instead by natural exposure to staphylococci and so the antibodies might have recognised the wrong ClfA epitopes or may have been of low affinity or avidity towards their target antigens.

Table 3

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo (n = 989)</th>
<th>INH-A21 (n = 994)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood infection with:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>50 (5%)</td>
<td>60 (6%)</td>
<td>0.34</td>
</tr>
<tr>
<td>CoNS</td>
<td>227 (23%)</td>
<td>247 (25%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>30 (3%)</td>
<td>33 (3%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Mortality</td>
<td>73 (7%)</td>
<td>57 (6%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Another Inhibitex product in the pipeline is a murine monoclonal antibody (mAb) 12-9 to ClfA. In pre-clinical studies, mAb 12-9 protected mice in a lethal sepsis model [13]. A humanised version of mAb 12-9, known as tefibazumab (Aurexis®), was produced by Inhibitex. In a rabbit model of endocarditis, two doses of tefibazumab (30 mg/kg) in combination with vancomycin resulted in fewer animals with bacteraemia and significantly fewer bacteria in the spleens and kidneys than in animals given vancomycin alone [14].

A phase II study of tefibazumab enrolled hospitalised patients with documented S. aureus bacteraemia [15] who received either a single tefibazumab dose plus standard therapy or standard therapy alone. To evaluate efficacy, a composite clinical endpoint was used consisting of a relapse of S. aureus bacteraemia, a complication related to the S. aureus bacteraemia or death. In the tefibazumab group, 2 (6.7%) of 30 patients reached the clinical endpoint compared with 4 (13.3%) of 30 patients in the placebo group (P = 0.455). Inhibitex is seeking a partner to support further clinical trials of tefibazumab.

5.2. AltaStaph

AltaStaph, produced by Nabi, is a hyperimmune polyclonal antibody preparation derived from healthy volunteers immunised with the bivalent StaphVAX preparation. In a phase II study, 206 low birthweight neonates were given AltaStaph or placebo on Days 0 and 14 [16]. The rates of S. aureus bacteraemia (ca. 3%) were nearly identical in both groups. Another phase II trial to evaluate AltaStaph enrolled 40 patients with documented S. aureus bacteraemia and persistent fever [17]. Five (24%) of 21 patients who received AltaStaph and standard therapy died compared with 2 (11%) of 18 in the group that received standard therapy alone (P = 0.42). These results confirm the premise that vaccine-induced antibodies to CPS and CPB are insufficient to reduce significantly S. aureus bacteraemia in at-risk populations.

5.3. Aurograb

NeuTec Pharma has identified an S. aureus ATP-binding cassette (ABC) transporter as a novel target for passive immunisation therapies against staphylococcal infection. This protein elicited an antibody response in patients who recovered from septicaemia and wound infections caused by an epidemic MRSA strain [18]. A significant correlation was reported between patient survival and serum IgG reactive with this immunodominant protein. Hyperimmune rabbit serum or recombinant antibodies reactive with certain epitopes of the ABC transporter were used to treat mice that had been challenged intravenously with an epidemic MRSA strain. Treated animals showed a ca. 1 log lower bacterial burden recovered from...
the liver, kidney and spleen after 24–48 h than control mice. NeuTec thus developed Aurograb, which is a single-chain variable antibody fragment against the S. aureus ABC transporter. In June 2006 the company completed a double-blind, placebo-controlled phase III clinical trial that compared the effects of Aurograb in combination with vancomycin versus vancomycin alone in the treatment of deep-seated MRSA infections. The results of this trial have not been released; in July 2006 NeuTec Pharma was taken over by Novartis.

5.4. Pagibaximab

Lipoteichoic acid (LTA) is a plasma-membrane-embedded glycolipid unique to Gram-positive bacteria. Biosynexus has developed a humanised mouse chimeric mAb against LTA called pagibaximab (BSYX-A110). The antibody is targeted at low birthweight infants for the prevention of bloodstream infections by S. aureus and CoNS [19]. In a phase II clinical trial, very low birthweight neonates were randomised to receive 60 mg/kg or 90 mg/kg pagibaximab or placebo on Days 0, 7 and 14. Preliminary results of this trial indicate that babies treated with the 90 mg/kg dose had fewer bacteraemias than the other groups [20]. Pagibaximab was licensed to GlaxoSmithKline in 2002, and in 2005 MedImmune licensed the rights to the product.

6. Pre-clinical development: surface-associated S. aureus antigens

6.1. Poly-N-acetylglucosamine

Poly-N-acetylglucosamine (PNAG), also known as polysaccharide-intracelluar adhesin, is a surface polymer produced both by S. aureus and S. epidermidis. PNAG promotes biofilm formation and enhances staphylococcal virulence in mouse infection models. The native form of PNAG is partially de-N-acetylated. Kelly-Quintos et al. [21] showed that only antibodies to the de-N-acetylated epitopes of PNAG (dPNAG) mediated antibody-dependent phagocytic killing of S. aureus by human neutrophils. In a follow-up study, mice were passively immunised with immune or non-immune serum and challenged intravenously with S. aureus 48 h later. Quantitative blood cultures revealed that mice given dPNAG antibodies had between 54% and 91% fewer S. aureus in their blood than mice given normal rabbit serum [22]. Antibodies to the native (acetylated) PNAG conjugate were ineffective in clearing bacteraemia in mice. Because dPNAG is preferentially retained on the bacterial cell surface, antibodies to dPNAG may be more effective in achieving protection than PNAG antibodies. Moreover, in human sera, levels of antibodies to dPNAG correlated with levels of in vitro opsonophagocytic killing [21].

6.2. Multicomponent S. aureus adhesin vaccine

Stranger-Jones et al. [23] systematically evaluated 19 cell-wall-anchored S. aureus protein adhesins for their vaccine potential in mice. Immunised mice were challenged with S. aureus Newman and the bacterial burden in the kidneys was evaluated 4 days later. The authors identified four conserved antigens (IsdA, IsdB, SdrD and SdrE) that protected mice against S. aureus-induced renal abscess formation. The antigen IsdB, previously identified by Merck scientists as a protective cell–wall–associated staphylococcal antigen [7], and IsdA are involved in heme iron uptake [24]. SdrD and SdrE are cell-wall-anchored surface proteins with unknown functions. Rabbit antisera to each of the proteins was opsonic in an opsonophagocytic killing assay against a protein A-negative mutant of S. aureus Newman. A mixture of antibodies to the four recombinant proteins showed higher opsonic activity than any of the individual sera. Animals that were immunised with the combination of all four surface components and then challenged with strain Newman had 100% survival at 7 days (Fig. 3). In comparison, animals immunised with either phosphate-buffered saline (PBS) or one of the individual components had survival rates of 50–70%. Immunisation with the multivalent vaccine significantly reduced S. aureus-induced mortality in mice compared with PBS for four of five S. aureus clinical isolates [23].

6.3. Heteropolymers

A novel heteropolymer technology is being developed by Elusys Therapeutics to combat S. aureus bacteraemias. The product (ETI-211) consists of a mAb to S. aureus protein A linked to a mAb directed against the human complement receptor 1 (CR1). The concept promoted by Elusys is that blood-borne S. aureus, bridged by the bispecific mAb complex, binds to CR1 on erythrocytes and that this complex is taken up and destroyed by macrophages in the liver and spleen. Pre-clinical studies to demonstrate efficacy utilised transgenic mice expressing human CR1 on red blood cells. Mice pre-treated with the heteropolymers survived a lethal S. aureus challenge dose, in contrast to mice pre-treated with the protein A mAb alone. Therapeutic administration of the heteropolymer to infected mice resulted in more efficient clearance of the bacteria from the liver, kidneys and spleen than in control mice given PBS. Elusys has a research collaboration with Pfizer to evaluate heteropolymers targeting S. aureus.

7. Exotoxin vaccines

7.1. Alpha-haemolysin

Alpha-haemolysin is a secreted S. aureus protein that can cause pore formation within eukaryotic cells. Adlam et al. [25] first reported that immunisation with a toxoid prepared from alpha
toxin protected rabbits against the lethal gangrenous form of S. aureus mastitis but did not prevent abscess formation. Similarly, in an experimental model of S. aureus keratitis, rabbits actively immunised with an alpha-haemolysin toxoid showed less corneal pathology and epithelial erosion than rabbits given adjuvant alone [26]. However, there was no difference in the number of bacteria recovered from the infected corneas of immunised or control rabbits. Menzies and Kernodle [27] created a non-toxic and non-haemolytic alpha-haemolysin mutant toxin (H35L) by site-directed mutagenesis. Passive immunisation with rabbit anti-H35L serum protected mice from lethal challenge with purified alpha-haemolysin and against acute lethal challenge with a high-alpha-haemolysin-producing S. aureus strain.

The severity of an acute staphylococcal lung infection in mice has been shown to correlate with the levels of alpha-haemolysin produced by different S. aureus isolates [28]. Wardenburg and Schneewind [28] used the H35L alpha-haemolysin mutant protein to immunise mice and evaluated protection against S. aureus in a murine pneumonia model. Mice immunised with the H35L protein and then challenged intranasally with S. aureus showed reduced lethality (Fig. 4) compared with animals injected with PBS. Vaccine-induced protection correlated with reduced inflammation and destruction of lung tissue and a ca. 1 log decrease in bacterial counts in the lung tissue. Similarly, antibodies to the H35L protein administered intraperitoneally protected mice against lethality following intranasal challenge with S. aureus. Mice given antibodies to alpha-haemolysin showed a ca. 1 log decrease in S. aureus counts in the lungs compared with control mice given normal serum. Antibodies to alpha-haemolysin clearly play a role in neutralising the lethal effects induced by this toxin. The efficacy of alpha-haemolysin antibodies in modulating other types of staphylococcal infections remains to be determined.

### 7.2. Superantigens

*Staphylococcus aureus* can secrete a wide variety of superantigen exoproteins, including toxic shock syndrome toxin-1, as well as ca. 15 different enterotoxins, and clinical isolates vary markedly in their production of these proteins. Superantigens act as potent oligoclonal T-cell activators, stimulating a massive release of pro-inflammatory cytokines. Aerosol exposure of non-human primates to staphylococcal enterotoxin B (SEB) results in gastrointestinal symptoms, lethargy, shock and death. Mutant forms of the superantigenic proteins that are devoid of their biological properties can be used as vaccines to elicit antibodies that neutralise the native toxin molecules. Importantly, when monkeys were actively immunised with a proteasome–SEB toxoid vaccine, all of the animals were protected against severe symptoms and death due to aerosolised SEB intoxication [29]. Passive immunisation with antibodies to SEB before or 4 h after aerosol exposure to SEB provided similar protection to rhesus monkeys [30]. Non-toxic derivatives of the staphylococcal superantigens may be useful vaccine candidates for either active or passive protection strategies against the use of aerosolised superantigens in biological warfare.

### 8. Antibodies to block S. aureus virulence

Vaccines that target bacterial virulence have the potential of debilitating the microbe such that the host immune system can more easily eradicate it. The major global regulator of virulence in *S. aureus* is the accessory gene regulator (agr), which modulates bacterial physiology and virulence factor expression through quorum sensing mediated by the secretion of small cyclic autoinducing peptides (AIPs). *Staphylococcus aureus* strains can be classified into four agr groups, and AIPs from the different groups inhibit agr expression by members of the heterologous groups. Park et al. [31] described the preparation of a mAb to AIP-4 that reduced the expression of RNAIII (the effector molecule of the agr locus) and alpha toxin. The AIP-4 mAb increased protein A expression and biofilm formation in an AIP subgroup-specific manner, but it did not affect bacterial growth in vitro. Mice passively immunised with the AIP-4 mAb were protected from a lethal intraperitoneal dose of *S. aureus* (Fig. 5), whereas mice given a control mAb were not protected. Likewise, mice challenged with *S. aureus* mixed with Cytodex® beads and 0.6 mg of the AIP-4 mAb (but not the control mAb) were protected from subcutaneous abscess formation.

Whether disruption of quorum sensing signalling will be beneficial against other *S. aureus* strains and other AIP groups is unknown. Since agr negatively regulates staphylococcal biofilm formation and the production of many surface adhesins, antibodies to AIPs may not be beneficial against all manifestations of *S. aureus* disease. Nonetheless, this conceptual approach of targeting *S. aureus* virulence factors is intriguing and has potential to aid in the control of acute infections caused by multidrug-resistant staphylococcal strains.

### 9. A vaccine to prevent nasal colonisation

The primary niche for *S. aureus* in humans is the nares, and nasal carriage is a documented risk factor for staphylococcal infection. Systemic *S. aureus* infections might be reduced by eliminating nasal carriage, since the source of ca. 80% of *S. aureus* bacteraemias is endogenous in origin. Mupirocin is effective in decolonising nasal carriers, although the emergence of mupirocin resistance in *S.
Conjugate vaccines. Serotypes following systemic immunisation with relevant capsule vaccination in a rodent model of nasal carriage [33]. Mice immunised intranasally with killed S. aureus colonisation than control animals. A ClfB mAb inhibited systemic or intranasally with ClfB demonstrated lower levels of and IsdH were high in to identify immunogenic proteins. Serum antibody levels to IsdA expression libraries with sera from infected and uninfected patients compared with a control mAb.

Binding to mouse cytokeratin 10 and reduced nasal colonisation of clinical isolates should be surface exposed, expressed by the majority of isolates belonging to diverse lineages, and show minimal serological variability among strains. Candidate antigens should elicit antibodies that promote opsonophagocytic killing in vitro by human neutrophils, elicit antibodies to block staphylococcal adherence and/or biofilm formation, and neutralise toxic S. aureus exoproteins. The accessibility of S. aureus surface antigens has important implications for vaccine development since optimal targets for immunisation should be surface exposed. For example, ClfA and CP are both expressed by S. aureus in post-exponential growth, but CP production at least partially masks cell-wall-associated ClfA and prevents it from binding to fibrinogen [35]. If an antibody to a surface antigen is masked by CP, it will not retain functionality (mediate opsonisation or block attachment). Vaccine efficacy would need to be tested in diverse models of S. aureus infection such as endocarditis, bacteraemia, pneumonia and wound infection.

Evaluation of the results of the clinical and pre-clinical studies suggests several good candidate vaccine antigens: CP, CP8, PNA, cell-wall-anchored proteins (ClfA and IsdB) and alpha-haemolysin H135A. We would argue for development of a multicomponent staphylococcal vaccine that includes at least one surface antigen (such as a fibronectin-binding protein A or IsdB) that is expressed during the exponential phase of bacterial growth. Antigen expression in vivo and under different growth conditions and media should also be explored.

The patients in most need of a staphylococcal vaccine comprise a population whose immune systems may not adequately respond to immunisation. For example, haemodialysis patients are at persistently elevated risk for S. aureus bacteraemia. However, the immune response to vaccination may be suboptimal in this cohort, as was seen with StaphVAX, hepatitis B and influenza vaccine studies. Likewise, premature neonates are susceptible to staphylococcal infections. Owing to their poorly developed innate immunity, passively administered antibodies alone may be insufficient to protect these babies. Targeting otherwise healthy individuals, such as those undergoing elective surgical procedures, may be the best way to demonstrate vaccine efficacy in clinical trials. Such trials may be essential to demonstrate proof of principle, i.e. that a protective S. aureus vaccine can be formulated. However, such a trial will not solve the greater problem of protecting compromised hosts from life-threatening S. aureus infections.

In developing an S. aureus vaccine, both active and passive approaches should be pursued, as these are not mutually exclusive and may very well turn out to be complementary. For severe S. aureus infections such as endocarditis, passive antistaphylococcal immunisation could be used as an adjunct to antibiotics. Eradication of nasal carriage by treatment with mupirocin, followed by administration of a vaccine that would prevent re-acquisition of S. aureus nasal colonisation, might reduce serious staphylococcal infections in certain at-risk populations. Recently discovered proteins and polysaccharides that may be critical targets for protective immunity against S. aureus raise the prospect that an effective staphylococcal vaccine may be developed.

**Funding:** Cangene Corporation (not vaccine-related) and discretionary funds.

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**Table 4**

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<thead>
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<th>Question</th>
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<tbody>
<tr>
<td>Can S. aureus infections be prevented by vaccination?</td>
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<tr>
<td>Who should receive the S. aureus vaccine?</td>
</tr>
<tr>
<td>What are the measurable correlates of protective immunity?</td>
</tr>
<tr>
<td>What are the appropriate antigens for inclusion in a multicomponent staphylococcal vaccine?</td>
</tr>
<tr>
<td>Can a multicomponent vaccine address the protean clinical manifestations of S. aureus disease?</td>
</tr>
<tr>
<td>Can one expect a vaccine to protect against an infection involving a prosthetic device?</td>
</tr>
<tr>
<td>Do the animal models chosen for pre-clinical studies reflect the pathogenesis of human disease?</td>
</tr>
<tr>
<td>Will a vaccine that reduces S. aureus nasal colonisation reduce staphylococcal infections?</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Effect of passive intraperitoneal immunisation with 1 mg of a monoclonal antibody (mAb) to autoinducing peptide 4 (AIP-4) on mice challenged with a lethal intraperitoneal inoculum of Staphylococcus aureus RN4220. Mice given phosphate-buffered saline (PBS) or an isotype-matched mAb succumbed to the infection. Reproduced from Park J, Jagasia R, Kaufmann GF, et al. Infection control by anti-body disruption of bacterial quorum sensing signaling. Chem Biol 2007;14:1119–27 with permission. Copyright 2007 Elsevier.
Competing interests: None declared.
Ethical approval: Not required.

References


