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Antistaphylococcal Vaccines and Immunoglobulins

Current Status and Future Prospects

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Abstract

Staphylococci are among the most frequently encountered pathogens in both the inpatient and the outpatient setting. Management of infections caused by these organisms is complicated by the increasingly common resistance of staphylococcal pathogens to commonly used antibacterials. As a consequence, novel approaches to prevention and treatment are urgently required. Such approaches include the development of vaccines and immunoglobulin preparations targeted at virulence factors expressed *in vivo* by staphylococci. This article reviews the biopharmaceutical progress made to date in this field and suggests approaches to further progress.

Staphylococcus aureus is among the most frequently encountered human pathogens, affecting the previously healthy in addition to the unwell. Furthermore, *S. aureus* is increasingly resistant to available β-lactam antibacterials in both the healthcare and the community setting. The annual incidence of invasive infections as a result of this pathogen has been reported to be 28.4 per 100 000 population in Canada.[1] The National Nosocomial Infection Surveillance programme reported meticillin resistance in 31.1% of *S. aureus* isolated from patients in hospital outpatient areas, 46.0% from non-intensive care unit (ICU) inpatient areas, and 52.9% from ICU patients in 1998–2004 in the US.[2] The Surveillance and Control of Pathogens of Epidemiologic Importance study reported that the overall incidence of *S. aureus* bloodstream infection in 49 US hospitals was 10.2 per 10 000 admissions and that the proportion that were meticillin resistant increased from 22% in 1995 to 57% in 2001.[3] The rapid emergence of community-acquired meticillin-resistant *S. aureus* (CA-MRSA) has also been remarkable. Prospective surveillance in Minnesota, Atlanta and Baltimore (US) in 2001–2 found that 8–20% of *S. aureus* isolates from individuals with community-onset infection were CA-MRSA, with an annual incidence of infection ranging from 18.0 to 25.7 cases per 100 000 population.[4] In addition to the problem of resistance to available β-lactam antibacterials, the emergence of reduced susceptibility to vancomycin further clouds our therapeutic horizon.[5]

Coagulase negative staphylococci are the most frequent cause of nosocomial bloodstream infection in the US, accounting for almost one-third of

cases.[3] They are the most prevalent pathogens causing late-onset sepsis in neonates, with *S. epidermidis* being responsible for a large proportion.[6] In one prospective study,[7] 16 (20%) of 82 infants admitted to an ICU nursery developed sepsis due to *S. epidermidis*; 81% of the episodes occurred in infants weighing <1000g.

While a number of antistaphylococcal antibacterials have recently been introduced and others are in development, the ever-increasing antimicrobial resistance of *S. aureus* demonstrates a need for novel preventive and therapeutic approaches. These include the development of biological agents, including vaccines and immunoglobulins that target virulence factors. Although the list of putative staphylococcal virulence factors is extensive, only a few have been targeted to date.

1. Some Currently Targeted Virulence Factors

1.1 Cell Envelope and Capsule

An *in vivo* expression system identified a total of 60 antigenic candidate antigenic proteins in *S. aureus*, most of which are either located or predicted to be located in the cell envelope or predicted to be secreted.[8] In addition to these, an important non-protein virulence factor is the antiphagocytic polysaccharide capsule. Strain differences in the capsule are the basis of a serotyping system.[9] Capsule production is under the positive control of the global regulator, *agr*, and is maximal during post-exponential growth. Its *in vitro* expression is markedly altered by a variety of ambient conditions, a factor that may account for variable results in the detection of capsular production *in vivo*.[9] Two *S. aureus* capsular types that are structurally similar, yet antigenically distinct, types 5 and 8, together account for serotypes causing most infections with this organism.[9] In a recent report from a single institution, capsular types 5 and 8 together accounted for 92% of 234 clinical *S. aureus* isolates, with type 336 accounting for the remaining 8%.[10] Antibodies to type 336 (which represents a cell wall polysaccharide) are cross-reactive with the majority of *S. epidermidis* isolates.[11]

The microbial surface components recognising adhesive matrix molecules (MSCRAMM) are important *S. aureus* virulence factors that are involved in binding of the organism to human extracellular matrix proteins and to foreign material, such as vascular catheters.[12] Among the MSCRAMM of *S. aureus* are clumping factors A (ClfA)[13] and B (ClfB) that bind to fibrinogen, the fibronectin-binding proteins (Fnbp), FnBpA and FnBpB, and the collagen-binding protein Cna. ClfA, present in >98% of strains of *S. aureus,*[14] inhibits phagocytosis by human polymorphonuclear leucocytes, both in the presence and absence of fibrinogen.[15] ClfB promotes attachment to human nasal epithelial cells by binding cytokeratin-10.[16] The collagen-binding adhesin Cna, has been demonstrated to contribute to the virulence of *S. aureus* in mice.[17] (Ser-Asp dipeptide repeat G [SdrG]) is a fibrinogen binding MSCRAMM present on the majority of *S. epidermidis* strains.[14]

S. aureus, like other Gram-positive bacteria, contains in its cell envelope surface-exposed teichoic acids, polymers of polyglycerol or polyribitol phosphate, with attached *N*-acetylglucosamine and D-alanine.[18] Teichoic acid bound to cell membrane lipid is referred to as lipoteichoic acid. The strong positive charge of these molecules is reported to provide the organism protection from host cationic antimicrobial peptides.[19] Teichoic acids mediate interaction with human nasal epithelial cells,[20,21] play a role in biofilm formation,[22] and are important as virulence factors in experimental murine models of *S. aureus* infection.[21]

Adenosine triphosphate-binding cassette (ABC) transporters facilitate the movement of a variety of molecules across cell membranes of bacteria.[23,24]

Substrates actively conveyed into the cell include nutrients and osmoprotectants, whereas those extruded include elements of the bacterial cell surface (e.g. capsular polysaccharides), toxins and antibacterials.[23] As a consequence, ABC transporters play a number of critical roles in microbial metabolism, virulence and antibacterial resistance.

1.2 Exotoxins

A large proportion of putative virulence factors in *S. aureus* are exoenzymes and toxins.[25] Among the toxins are a number that act as superantigens; staphylococcal toxic shock syndrome toxin-1 (TSST-1) is an important example.[26] Another class of toxins are synergohymenotropic molecules such as the Panton-Valentine leukocidin (PVL), which lyses neutrophils and monocytes.[27] The presence of the genes encoding PVL, while generally absent from classical hospital-acquired strains of MRSA, are present in most CA-MRSA in the US and Europe. The presence of the PVL gene has been associated with increased severity of skin infection and of pneumonia.[28]

2. Active Immunisation

2.1 Targeting *Staphylococcus aureus* Capsular Polysacccharide: StaphVAX®

The immunogenicity of polysaccharide antigens, which is generally poor, may be improved by their covalent coupling to protein carriers. Coupling of capsular type *S. aureus* polysaccharides 5 and 8 to nontoxic (because of a single amino acid deletion) recombinant exotoxin A from *Pseudomonas aeruginosa* enhances their immunogenicity in mice and humans[29-31] and results in a protective vaccine in a number of murine models of *S. aureus* infection.[9]

StaphVAX® [1] is a bivalent vaccine containing *S. aureus* capsular polysaccharides 5 and 8 conjugated to nontoxic recombinant *P. aeruginosa* exotoxin. A phase I trial demonstrated induction of both IgM and IgG antibody to capsular polysaccharide, but no booster response to a second injection 6 weeks after the first.[30] In a phase II trial in patients receiving chronic peritoneal dialysis, the vaccine dose selected based on results from the phase I trial proved to be poorly immunogenic. A second phase II trial demonstrated significant increases in antibody levels after a two-dose immunisation schedule in patients with end-stage renal disease, but this response was only approximately one-half that observed in healthy volunteers,[32] an observation that led to an increased dose of vaccine in subsequent trials.

A large, phase III trial evaluated the safety and efficacy of StaphVAX® in the prevention of *S. aureus* bacteraemia in patients with end-stage renal disease undergoing chronic haemodialysis.[33] While there was no statistically significant decrease in the incidence of bacteraemia between weeks 3 and 54, vaccine efficacy between weeks 3 and 40 was 57% ($p = 0.02$). The diminution in efficacy after 40 weeks was paralleled by a decline in antibody to capsular polysaccharide. Analysis of these results concluded that an antibody concentration of approximately 80 μg/mL represented a reasonable estimate of the amount required for protective efficacy.

These results were considered sufficiently encouraging to justify the performance of a second larger phase III trial involving 3600 haemodialysis patients. Unfortunately, this trial failed to meet its primary endpoint and, in November 2005, further development of StaphVAX® was put on hold.[34] The sponsor, Nabi Biopharmaceuticals, subsequently announced the results of an outside advisory panel's assessment of the development programme on 21 March 2006.[35] The vaccine used in the confirmatory phase III trial had been produced by a contract manufacturer and proved to elicit antibod-

1 The use of trade names is for product identification purposes only and does not imply endorsement.

ies that were functionally inferior to those produced in response to vaccine used in earlier studies and manufactured by Nabi Biopharmaceuticals. In animal studies, human antibodies raised in response to the newer preparation protected only 30–50% from challenge with *S. aureus* capsular type 8, compared with 100% protection with older preparations. It was also concluded (although no supporting information is provided) that the "virulence of the bacteria may have become even more significant in the period since the first phase III clinical study was conducted in 1999 and 2000" and the combination of this factor with the production of less effective antibody accounted for the negative results.[35] See table I for a summary of active and passive immunisation.

3. Passive Immunisation

3.1 Targeting *S. aureus* Capsular Polysacccharide: Altastaph®

Altastaph® is a hyperimmune polyclonal human immune globulin preparation obtained from healthy volunteers vaccinated with the StaphVAX® and thus enriched for antibody to *S. aureus* capsular polysaccharides types 5 and 8.[36] Altastaph® administration had been safely demonstrated in premature low birthweight infants, a population with a high incidence of staphylococcal infections.[40,41] In a small, randomised, placebo-controlled, trial in 40 patients with *S. aureus* bacteraemia that was persistent or in whom fever persisted, Altastaph® administration resulted in high levels of opsonising antibody in serum and with shorter duration of bacteraemia and fever, although neither of the latter were statistically significant.[42] A larger placebo-controlled trial involving 206 very low birthweight infants confirmed the ability two infusions of Altasaph® to achieve high concentrations of antibody to *S. aureus* types 5 and 8, but was unable to demonstrate efficacy: three episodes of *S. aureus* bacteraemia occurred in the 56 Altastaph® recipients and three in the placebo recipients.[41] However, the failure of the confirmatory phase III trial of StaphVAX® has led to a reassessment of the Altastaph® programme, leading to its interruption.[35]

3.2 Targeting *S. aureus* Clumping Factor A: INH-A21 and Tefibazumab

3.2.1 INH-A21

Passive immunisation with anti-ClfA antibodies enhances survival in mice challenged with *S. aureus*.[13] Administration of SA-IVIG, a polyclonal immunoglobulin preparation derived from plasma donors with naturally occurring high IgG antibody titres to ClfA and with demonstrated opsonic and antiadhesion properties, improved clearance of *S. aureus* from blood and vegetations in a rabbit model of MRSA bacteraemia when added to vancomycin therapy.[43]

INH-A21 (Veronate®) is a polyclonal human donor-selected immunoglobulin preparation based on SA-IVIG that contains high concentrations of antibody to the *S. epidermidis* MSCRAMM, SdrG, as well as to ClfA of *S. aureus*.[44,45] It has also been found to cross-react with antigens of *Candida albicans*.[45] INH-A21 was protective in a suckling rat model of *S. epidermidis* infection and as prophylaxis and therapy in rabbits with experimental endocarditis due to either *S. epidermidis* or MRSA.[44] Administration of up to four doses of INH-A21 750 mg/kg to very low birthweight human infants was well tolerated and, when compared with placebo administration, associated with reductions in episodes of *S. aureus* sepsis, candidaemia and mortality; none, however, reached statistical significance.[14,46]

This led to the performance of a double-blind, placebo-controlled phase III trial that enrolled 2017 very low birthweight infants. As many as four doses of INH-A21 or placebo were administered on days 1, 3, 8 and 15. The study had a 90% power to detect 50% reduction in late-onset sepsis due to *S. aureus*.

Table I. Active and passive immunisation

Target	Product	Pharmaceutical company	Goal	Status	Reference
Active immunisation: vaccine					
Staphylococcus aureus capsular polysaccharide types 5 and 8	StaphVAX® (*S. aureus* conjugate)	Nabi Biopharmaceuticals	Prevention of infections as a result of *S. aureus*	On hold; expansion to a multivalent vaccine to also include *S. aureus* 336 and *S. epidermidis* PS-1 and possibly *S. epidermidis* GP-1 and PVL	36
Passive immunisation: immunoglobulin preparations					
S. aureus capsular polysaccharide types 5 and 8	Altastaph® (*S. aureus* immunoglobulin)	Nabi Biopharmaceuticals	Prevention of infections as a result of *S. aureus* in at-risk neonates	On hold, pending increased multivalency of StaphVAX® to include *S. aureus* 336 and *S. epidermidis* PS-1 and possibly *S. epidermidis* GP-1 and PVL	36
S. aureus ClfA, *S. epidermidis* SdrG	INH-A21 (Veronate®)	Inhibitex Inc.	Prevention of staphylococcal sepsis in very low birthweight infants	Failed phase III trial; development suspended	37
S. aureus ClfA	Aurexis® Tefibazumab (MAb T1-2)	Inhibitex Inc.	Adjunctive therapy of *S. aureus* bacteraemia	Completed phase II	37
S. aureus ABC transporter GrfA	Aurograb® (anti-MRSA monoclonal antibody)	Neutec Pharma plc	Adjunctive therapy of *S. aureus* bacteraemia	Phase III	38
Lipoteichoic acid	Pagibaximab (BSYX-A110)	Medimmune (under license from Biosynexus and GlaxoSmithKlein)	Prevention of staphylococcal infection	Completed phase IIa	39

ABC = adenosine triphosphate-binding cassette; **ClfA** = clumping factor A; **MRSA** = meticillin-resistant *S. aureus*; **PVL** = Panton-Valentine leukocidin; **SdrG** = Ser-Asp dipeptide repeat G.

On 3 April 2006, Inhibitex reported that INH-A21 therapy failed to meet the primary endpoint of prevention of *S. aureus* infections and that, in addition, there were "no measurable effects or trends in favour of Veronate for the primary or any of the secondary endpoints".[37] The sponsor announced on 28 April 2006 that it was reviewing all its data, but that it had "suspended future collections of all plasma used to manufacture the current donor-selected immune globulin form of Veronate®".[37,47]

3.2.2 Tefibazumab

Tefibazumab (Aurexis®; MAb T1-2), a modification of murine MAb 12-9, is a humanised murine IgG1κ monoclonal antibody with high affinity for ClfA that has been demonstrated to inhibit *in vitro* binding of *S. aureus* to human fibrinogen and to improve survival in a murine model of MRSA infection.[48] The amino acid sequence of its variable antigen-binding region is >98% human and <2% murine.[49] Tefibazumab administered prior to infection provided protection against intravenous challenge with MRSA in a murine model of septicaemia.[50,51] It was also effective in combination with vancomycin as therapy of MRSA endocarditis in a rabbit model.[50,51] Tefibazumab was well tolerated when administered as a single infusion of various doses in a phase I clinical trial in healthy volunteers in whom it had a plasma half-life of approximately 22 days.[49]

A randomised, double-blind, phase II trial evaluating the safety and pharmacokinetics of tefibazumab enrolled 60 patients *S. aureus* bacteraemia.[52] Patients, all of whom received standard of care antibacterial therapy, were randomised to also receive a single dose of tefibazumab or placebo. The plasma half-life of the monoclonal antibody was 18 days; exposure was less than that seen with equivalent doses in healthy volunteers. Four patients receiving antibacterials alone reached a predetermined composite adverse outcomes endpoint, compared with two who also received tefibazumab; four patients in the former group and none in the latter experienced progression in the severity of sepsis.

A phase II trial is currently evaluating the safety and pharmacokinetics of tefibazumab in children with cystic fibrosis with persistent respiratory tract *S. aureus* colonisation[53]

3.3 Targeting an Adenosine Triphosphate-Binding Cassette Transporter: Aurograb®

Evaluation of serum from patients with infection as a result of an epidemic MRSA strain led to the identification of an immunodominant staphylococcal antigen with seven epitopes and a derived amino acid sequence showing homology with ABC transporters.[54] Furthermore, recombinant antibody targeting predicted epitope sequences of the transporter were associated with a reduction in bacterial load in a rabbit model of MRSA infection.[54] Aurograb®, a human recombinant single chain antibody fragment (scFv) that binds to the ABC transporter, GrfA, an immunodominant surfact antigen, was demonstrated to have *in vitro* activity against multiple strains of *S. aureus*, including vancomycin-intermediate *S. aureus* (VISA) and epidemic strains of MRSA, and to be synergistic with vancomycin.[38] A phase IIb trial completed in June 2003, demonstrated tolerability.[55] Phase III, double-blind, placebo-controlled trials in adults with deep staphylococcal infection are evaluating the efficacy of treatment with vancomycin alone or vancomycin plus Aurograb®.[55]

3.4 Targeting Teichoic Acid: Pagibaximab

Pagibaximab (BSYX-A110) is a humanised mouse chimerical monoclonal antibody against lipoteichoic acid with opsonic activity against staphylococci that is reported to provide protection in rodent models of infection with either *S. aureus* or *S. epidermidis*.[56,57] Phase I–II studies in adults and in very low birthweight infants demonstrated that its

administration was well tolerated and resulted in high titres of opsonising IgG1 antibody, with a plasma half-life of 30 ± 10.4 days in healthy adults.[58,59]

In a phase II study, 88 very low birthweight neonates were randomised to receive pagibaximab (60 mg/kg or 90 mg/kg) or placebo in three doses 7 days apart.[60] Pagibaximab well tolerated with plasma concentrations >500 μg/mL maintained for 3 weeks after the last 90 mg/kg dose. No cases of staphylococcal sepsis occurred in the group that received doses of pagibaximab 90 mg/kg, compared with a 20% incidence in those who received doses of pagibaximab 60 mg/kg and 13% in placebo recipients. Estimated or observed pagibaximab levels were <500 μg/mL at the time of staphylococcal sepsis in all cases but one.

4. Conclusion and Future Considerations

Staphylococci have an enormous evolutionary head start on humans, having appeared on earth more than a billion years ago. It is not surprising that they, particularly *S. aureus*, have become such successful human pathogens. The availability of an increasing number of genomic sequences of *S. aureus* strains provides us with a growing insight into the means by which this organism has been so successful in developing its extensive ecological niche and in causing disease in humans. Despite these advances, it is obvious that our understanding is incomplete. The lack of success of StaphVAX® and INH-A21 in phase III trials and the suspension of production of Altastaph® in response to the failure of StaphVAX® are indications that the path to the development of successful vaccines or immunoglobulins for the prevention and treatment of staphylococcal infections is likely to remain an arduous one. Success in these endeavors will have to deal with the obvious redundancy in virulence factors in staphylococci, including their ability to persist intracellularly,[61] often as small colony variants,[62] to grow as a biofilm,[63] and the multiple mechanisms by which it evades mechanisms of innate immunity.[64]

The incorporation of multiple targets in future biologicals is one approach that is likely to be fruitful. In adopting this approach, Nabi Biopharmaceuticals has decided to proceed by developing a multivalent vaccine to include, in addition to *S. aureus* capsular polysaccharide types 5 and 8, *S. aureus* 336 and *S. epidermidis* PS-1. PS-1 antigen is found on approximately 60–70% of *S. epidermidis* strains.[11] Successful phase I trials have already been conducted with the latter two immunogens. The sponsor also plans to 'advance and fold in' additional antigens and immunogenic toxins. These may include *S. epidermidis* GP-1 and PVL. The multivalent vaccine will also lead to the development of a next-generation Altastaph®, which will be produced from plasma of volunteers immunised to antigens of *S. aureus* types 5, 8 and 336, as well as *S. epidermidis* PS-1.[35]

Among additional targets that may be considered are several MSCRAMMs. For instance, in separate investigations, specific DNA vaccines were able to elicit antibody responses to ClfA and to Fnbp in dairy cows.[65,66] Immunisation of mice with ClfB was associated with reduced nasal colonisation with *S. aureus*.[67] Vaccination of mice with recombinant fragments of Cna provided protection against otherwise lethal inocula of *S. aureus* and serum from rats immunised with these fragments was also protective.[68] Immunisation of mice with a Cna-FnBp fusion protein provided protection against challenge with *S. aureus*.[69] A number of exotoxins, such as TSST-1, are additional potential targets. Vaccination with modified nontoxic TSST-1 has been demonstrated to improve survival after experimental challenge of mice with *S. aureus*,[70] as has vaccination with a TSST-1 fusion protein.[71] Patients with low concentrations of serum antibody to TSST-1 are at increased risk of recurrence of staphylococcal toxic shock syndrome.[72] Commercially available

intravenous immunoglobulin preparations contain variable amounts of antibody, capable of neutralising TSST-1 and other superantigens, as well as PVL.[73,74]

Enhanced vaccine immunogenicity may be explored by incorporation of an additional carrier protein and exploration of the use of adjuvants.[75] Greater immuogenicity may help to overcome a central problem: patients who are in most need of successful vaccination, such as those with end-stage renal disease receiving chronic dialysis therapy, are those least likely to adequately respond. However, there are populations in need of protection from staphylococcal infection who are better able to respond to the vaccine, e.g. patients undergoing cardiovascular or orthopedic surgery,[75,76] and perhaps participants in contact sports. The importance of finding means of reducing the burden of *S. aureus* infections is demonstrated by the fact that, despite setbacks associated with previous attempts discussed here, a phase I trial of a new antistaphyloccal vaccine, 0657nl, whose composition has not been publicly disclosed, was initiated by Merck and Co. in March 2006.[77]

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