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Targeted Immunotherapy for Staphylococcal Infections

Focus on Anti-MSCRAMM Antibodies

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Abstract

Staphylococcal infections represent an enormous burden to the public health system in the US and worldwide. While traditionally restricted to the hospital setting, highly virulent strains have recently emerged that may cause severe, even fatal, disease in healthy adults outside healthcare settings. This situation, together with the increasing resistance to many antibacterials in a wide variety of staphylococcal strains, requires that vaccine development for staphylococcal diseases be re-evaluated. Finding a vaccine for staphylococci is not trivial, as protective immunity to staphylococcal infections does not appear to exist at a significant degree, which may be partly due to the fact that our immune system is in constant contact with staphylococcal antigens and many strains are commensal organisms on human epithelia. Furthermore, the most virulent species, *Staphylococcus aureus*, produces protein A, a powerful means to evade acquired host defense.

While two high-profile vaccine preparations have failed clinical trials within the last few years, promising results from novel approaches based on the combination of systematically selected antigens have been reported. These combinatory vaccines target microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), a family of bacterial proteins that bind to human extracellular matrix components. In addition, polysaccharide and other nonprotein antigens may represent suitable vaccine targets on the staphylococcal cell surface.

In an era of increasingly frequent cases of multiple antibacterial resistance and decreasing investment by pharmaceutical companies in the field of antibacterial drug development, vaccines represent an alternative for pathogens such as staphylococci, for which efficient vaccination was long deemed impossible. Vaccination has several advantages over antibacterial treatment; for

example, the possibility for prophylaxis and the lesser risk for the development of resistance. However, staphylococci have certain features that make the development of a vaccine difficult, and perhaps even impossible, as evidenced by the current lack of an antistaphylococcal vaccine available for clinical use. This article provides an overview of the past and present efforts to develop antistaphylococcal vaccines, relating the problems encountered in these efforts to the specific biology of staphylococci and the pathogenesis of staphylococcal infections.

1. Staphylococcal Infections

Staphylococcus aureus is a Gram-positive bacterium that transiently or permanently colonizes humans, mostly in the nares, axilla, genital, and anoperineal areas. It is a common source of food poisoning and may cause several severe diseases, including septic, arthritis, endocarditis, toxic-shock syndrome, and scalded skin syndrome. Furthermore, *S. aureus* is the leading pathogen involved with skin and soft tissue, lower respiratory tract, and bloodstream infections. Finally, together with coagulase-negative staphylococci, *S. aureus* represents the most frequent cause of hospital-acquired infections, which often involve biofilm formation on indwelling medical devices.[1]

Although staphylococcal infections are predominantly seen in young children, the elderly, and immunocompromised individuals, and have traditionally been exclusively nosocomial, highly virulent strains of *S. aureus* have recently emerged that may infect healthy adults outside the hospital setting.[2] These community-associated strains combine exceptional virulence with antibacterial resistance (community-associated methicillin-resistant *S. aureus* [CA-MRSA]) and pose a severe threat to the public health system in the US and elsewhere, second only to HIV/AIDS in scope and importance. Although CA-MRSA infections were initially restricted to certain high-risk groups, which included sports teams, prisoners, men who have sex with men, and children,[3-6] CA-MRSA infections now represent a genuine pandemic on a broad scale. A recent report describes CA-MRSA as by far the most frequent source of skin and soft tissue infections reporting to emergency departments in the US.[7] Almost all of the reported infections were due to sequence type USA300 and thus strongly clonally related.[8]

In *S. aureus* and coagulase-negative staphylococcal infections, resistance to methicillin and other antibacterials is common.[9,10] In many infections with highly resistant strains, vancomycin is the only antibacterial of last resort. High-level resistance to vancomycin has been reported in *S. aureus* in several cases.[11,12] However, since these cases were reported, we have fortunately not seen a rapid spread of vancomycin-resistant *S. aureus*. Nevertheless, the increase of MRSA and especially the occurrence of CA-MRSA urgently require novel strategies to combat staphylococcal infections.

2. Who Would Receive a Staphylococcus Vaccine?

Before exploring why it may be difficult to achieve protective immunity against *S. aureus* and other staphylococci, one should consider who could receive an antistaphylococcal vaccine and under which circumstances. This requires identification of populations at high risk for staphylococcal infection. Although the emergence of CA-MRSA may redefine risk groups, and more data regarding CA-MRSA epidemiology and the molecular basis of virulence must be awaited, there are some patients with clearly increased risk of staphylococcal infections who may benefit from receiving an antistaphylococcal vaccine. These include dialysis patients, patients with ventriculoperitoneal shunts, patients at risk of infective endocarditis, immunocompromised individuals such as AIDS patients, and residents of nursing homes. In addition, as colonization with *S. aureus* is a clear risk factor for infection,[13] one might also consider vaccinating healthcare personnel, who probably represent intermittent carriers for infectious strains. Finally, patients undergoing surgery are at a high risk for staphylococcal infection. Surgical site infection rates range from 0.4% to 5% and are mostly caused by staphylococci,[14] with *S. aureus* and coagulase-negative strains (mostly *S. epidermidis*) found in roughly equal numbers. It has been argued that active immunoprophylaxis might be indicated for the placement of intravascular (infection rate 0.6–0.9%) and prosthetic (infection rate 0.4–0.9%) devices, while passive immunoprophylaxis may be applicable for the insertion of central venous catheters (CVC), which is a frequent cause of bloodstream infection.[15] This immunoprophylaxis for CVC-related bloodstream infection may be indicated before or during insertion or while on mechanical ventilator support. However, similar to the treatment with antibacterials, biofilm formation may render immunoprophylaxis ineffective and, thus, early application before insertion appears most appropriate.

Whether a more universal immunization throughout the population makes sense depends on several factors. While such a broad-scale application may not be financially viable, there is some scientific support of this idea. Because *S. aureus* infection often originates from carrier colonization, eradication of colonizing *S. aureus* in those carriers may also significantly decrease *S. aureus* infection in the population. Furthermore, there are some rarer *S. aureus* infections in patients without predisposing risk factors, namely in those with trauma and infective endocarditis, which could certainly be decreased in number by eradicating

S. aureus or at least decreasing the frequency of *S. aureus* colonization. Finally, the spread of CA-MRSA infections definitely supports such broad-scale immunization. However, infection routes of CA-MRSA differ from the traditional ones, inasmuch as the role of nasal colonization in CA-MRSA is controversial and probably minor.[16,17] Thus, this hypothesis needs to be re-evaluated in the light of CA-MRSA epidemiology and transmission.

Immediate, albeit short-term, immunoprophylaxis using passive immunization may be indicated during emergency surgery and in premature and critically ill infants. Low-birthweight infants are at an extremely high risk; one in three infants with a weight <1 kg will develop a nosocomial infection. Coagulase-negative staphylococci are the most common causes of bloodstream infections treated in neonatal and pediatric intensive care units and significantly affect patient mortality and morbidity.[18] Furthermore, antibacterial treatment is often difficult in these cases, owing to methicillin resistance and biofilm formation, which are widespread in coagulase-negative staphylococci.[19]

3. Vaccines for Staphylococcal Infections: a Reasonable Approach?

Since staphylococci have been recognized as a cause of human diseases, and long before the antibacterial era, the development of an antistaphylococcal vaccine has been proposed to prevent and treat staphylococcal infections. However, staphylococcal infection does not cause protection in the long term and, accordingly, more simple approaches such as by whole-cell live or killed vaccines generally do not achieve substantial protection from *S. aureus* infection. This leaves open the question of whether staphylococci have increased protection from acquired immunity and, if so, what is the basis for this protection? In fact, more recent research on immune evasion mechanisms in staphylococci has provided more detailed insight into the basis of this phenomenon. *S. aureus* and coagulase-negative staphylococci produce extracellular capsule-like substances that are poorly immunogenic (although tested as antigens, see section 9) and provide protection from innate and acquired immunity. These include the polysaccharide capsule of *S. aureus*,[20] which differs in composition between subgroups, the glucosamine polymer polysaccharide intercellular adhesin (PIA), which is found in both *S. aureus* and coagulase-negative strains,[21,22] and the recently detected poly-γ-glutamic acid capsule substance (PGA), which is found only in coagulase-negative staphylococci.[23] Furthermore, several other substances, such as teichoic acids and DNA,[24,25] contribute to the formation of a slimy biofilm matrix, one function of which is to protect from host defenses.

A more specific means of protection from acquired host defense is *S. aureus* protein A, a well known molecule used in biotechnology laboratories because of its function in binding to the F_C portion of IgG antibodies.[26] During infection, this feature is believed to provide significant protection from host defenses, as the large number of surface-bound nonspecific IgG antibodies serves as a 'camouflage coat'. In animal models of infection, protein A contributes to survival in only a marginal manner, although it appears to have important additional functions in virulence, such as a pro-inflammatory role in airway epithelia.[27] However, as stressed by Projan et al.,[15] the most interesting question, whether protein A may be of much more value for the bacteria in a vaccinated host, has not been addressed experimentally. The fact that most *S. aureus* strains express protein A, albeit in greatly varying amounts, supports this hypothesis. On the other hand, protein A as a common surface-exposed antigen may represent an interesting target for vaccine development. However, in an earlier study using a rat infant model, anti-protein A antibodies were not protective.[28]

Finally, several problems with antistaphylococcal immunoprophylaxis originate from the fact that staphylococci are frequent commensal organisms of humans. As one consequence, animal protection models have only limited value for antistaphylococcal vaccine development, as these animals, in contrast to humans, usually have not been in constant contact with staphylococcal antigens. Thus, only at the stage of clinical trials is really valuable information on the efficacy of antistaphylococcal vaccines obtained. Furthermore, the eradication of a common part of the human microflora may lead to severe disturbances of the physiological balance on human epithelia. While eradication of *S. aureus* may not pose such a problem, as we know that many healthy humans do not carry *S. aureus* transiently or permanently, this is especially valid for coagulase-negative strains such as *S. epidermidis*, which is a ubiquitous colonizer in humans.

An interesting recent study has compared the antibody repertoires in healthy *S. aureus* carriers with those in acutely infected patients and found that, whereas for some antigenic proteins, specific antibodies were missing in infected patients, high-titer antistaphylococcal antibodies are stable for years in healthy individuals.[29] There was considerable heterogeneity in antibody titers among the tested individuals, and only for some tested surface proteins were titers significantly higher in the infected group (SdrD, HarA, FnbpA, enolase, EbpS, and SA0688). Although the lessons to be learned from this study are mainly that an investigation of the individual's antibody titers to staphylococcal antigens is warranted upon hospitalization, the results might explain why some people become infected by *S. aureus* whereas others do not. Although innate host defense probably plays a more crucial role

for these host-to-host differences in susceptibility to staphylococcal infection,[30] this study gives some hope as to the general feasibility of the development and efficacy of an antistaphylococcal vaccine.

However, all antistaphylococcal vaccines developed thus far have failed in clinical trials. An experimental hyperimmune IgG preparation called Veronate® [1], developed by Inhibitex (Alpharetta, GA, USA), is based on selected sera from patients with high titers to the staphylococcal surface protein clumping factor A (ClfA). While results from phase II trials had been promising,[31] target endpoints were not reached during phase III trials. Active immunization using a *Pseudomonas* exotoxin A-coupled type 5 and type 8 *S. aureus* capsular polysaccharides (StaphVax®, Nabi Biopharmaceuticals, Boca Raton, FL, USA)[32,33] also failed during phase III clinical trials. In this case, there was no significant difference between vaccinated and unvaccinated groups. One prominent problem with the StaphVax® vaccine may have been the emergence of new capsular types during the trial and the problem of capsular escape using different capsular structures in *S. aureus* in general.

Summing up, while there are some potentially severe problems with regard to the development and use of an antistaphylococcal vaccine, the spread of antibacterial resistance and novel threats such as the rise of CA-MRSA certainly warrant a closer look at alternatives to antibacterial therapy.

4. Selection of the Best Antigen Targets

What are the best antigens for an antistaphylococcal vaccine? This is undoubtedly the central question in vaccine development. Antigens are being selected because they (i) are surface located, as surface proteins have proven more antigenic; (ii) are expressed at high levels *in vitro* and when experimentally approachable, *in vivo*; and (iii) play a role in virulence. With regard to the latter, while virulence factors do not necessarily represent the best antigens, antibodies directed against virulence factors may not only neutralize the virulence function of those factors, but may also prove more effective, because the organism depends on their expression during infection. However, completely indispensable virulence factors are not known in *S. aureus* or coagulase-negative staphylococci, and there is frequent functional redundancy. Thus, this approach has only limited applicability for staphylococci. So far, vaccine development for staphylococcal infections has mostly focused on surface-exposed antigens, including surface polysaccharides and proteins. None of these is indispensable for staphylococcal survival in the human host and, thus, several researchers have tried to combine several antigens in antistaphylococcal vaccines. This has been performed mostly in non-systematic attempts based on the criteria outlined above. However, more recently, extensive systematic approaches have been taken to select a combination of antigens that show high *in vitro/in vivo* immunogenicity.

5. Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs)

Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) are a class of surface molecules expressed by bacteria that bind to components (such as fibrinogen, fibronectin, vitronectin, laminin, elastin) of the human extracellular matrix, a complex 3-dimensional network surrounding cells in mammalian tissue.[34] The term MSCRAMM was coined in 1994, and in its stricter sense comprises the following surface proteins in *S. aureus*:[35] the clumping factors or fibrinogen-binding proteins ClfA and ClfB, the collagen-binding protein Cna, the fibronectin-binding proteins FnbpA and FnbpB, the bone sialoprotein-binding Bbp, and several members of the serine-aspartate repeat-containing Sdr family with yet undefined ligands. Sdr proteins are also found in *S. epidermidis*, and the SdrG protein has been shown to bind fibrinogen.[36,37] MSCRAMMs have a common structural organization: the N-terminal signal sequence is followed by the relatively conserved 'A' region, which comprises IgG fold domains and often the ligand-binding domain. Towards the C-terminus, there is more variety, and the B, C, and D domains may or may not be present in different members of the family. The C-terminal domain also often comprises repeat regions. Finally, at the extreme C-terminus, there is a cell wall-spanning domain followed by an LPXTG amino acid sequence motif and a membrane-spanning domain. The LPXTG motif is required for sortase-catalyzed covalent linkage to the peptidoglycan, but proteins that lack the LPXTG motif may still be surface-located, merely depending on the cell wall and cell membrane integration domains.

In a broader sense, the following proteins also belong to the MSCRAMM family, although they do not share the typical structural composition of the aforementioned proteins:[35]

- protein A, which binds not only the F_C part of IgG molecules, but also the extracellular matrix component von Willebrand factor;
- the major histocompatibility complex (MHC) class II analogous protein (Map; also known as extracellular adherence protein, Eap), which contains repeats that mimic the peptide binding groove of the β chain of the MHC class II proteins[38] and which interacts with a series of ligands;

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

- the autolysin/adhesin family of noncovalently anchored surface proteins, which comprise *S. aureus* Atl and *S. epidermidis* AtlE and Aae, and also interact with several ligands, for example vitronectin;
- the collagen-binding lipase GehD of *S. epidermidis*, which, similar to the autolysins, has a double catalytic and binding protein function.[39]

In addition to their potential as antigen targets for vaccine development, MSCRAMMs and other surface proteins have long been recognized as targets for target-oriented drug development. Although not much evidence exists to support this hypothesis, it is commonly agreed that surface binding proteins such as MSCRAMMS play a vital role for the invading organism during the establishment of an infection, when adhesion to host tissues is important for pathogen survival. Later in the course of infection, MSCRAMM genes are believed to be downregulated, most notably by the quorum-sensing system *agr*.[40] This putative shift in surface protein expression *in vivo* is largely derived from *in vitro* results and awaits *in vivo* evidence. However, a more recent study has shown that the quorum-sensing system *agr* is upregulated relatively early during infection and maintained at a considerable expression level, except for a likely neutrophil-induced metabolic eclipse lasting for 2–3 days.[41] As a consequence, the temporal window for MSCRAMM expression appears rather narrow, and whether for vaccine or drug development, more detailed knowledge of *in vivo* pathogenesis events is crucial for the development of MSCRAMM-targeted therapeutics.

6. Anti-MSCRAMM Vaccines

Because of their function in virulence and location at the bacterial cell surface, MSCRAMMs and other surface proteins have been in the focus of antistaphylococcal vaccine development. Anti-MSCRAMM antibodies might be helpful in opsonization or the prevention of adhesion. In one study, a fragment spanning the A region of the collagen-binding protein Cna, containing the collagen-binding domain (CBD), was produced as a recombinant protein.[42] Mice that were vaccinated with this recombinant Cna portion (rCBD) had a significantly higher survival rate than control mice (87% vs 13%). Further, bacteria opsonized for phagocytic uptake with sera from rCBD-immunized animals were ingested more efficiently. Using a Cna fusion protein as a vaccine, significant protection from *S. aureus* challenge was achieved in a model of staphylococcal endocarditis.[43] Antibodies against recombinant Cna fragments were also used for passive immunization experiments and proved protective. In a similar study, a recombinant form of ClfA was used to vaccinate mice, which exhibited a reduced arthritic response.[44] With lethal doses of *S. aureus*, there was a significantly higher survival rate in the vaccinated mice (87% vs 53% in control mice). Further, anti-ClfA antibodies significantly reduced the development of experimental arthritis in passive immunization experiments. Flock's group engineered cow-pea mosaic virus as a vector to express a truncated fragment of the D2 unit of FnbpB and immunized rats.[45] This caused an increased antibody titer specific for the D2 subunit. In a coupled arthritis and endocarditis model, there was decreased colonization of the aortic valve but not of the joints, suggesting that the humoral response did not provide significant protection from the *S. aureus* challenge, but could decrease the dissemination to other sites of infection. A more recent study conducted by Merck, Intercell, and Vaxgen investigated the vaccine potential of the ubiquitous iron-sequestering protein IsdB (iron surface determinant B).[46] The preparation was highly immunogenic in mice and rhesus macaques and provided significant protection in a murine sepsis model. Furthermore, Zhou et al.[47] produced a fusion protein of Cna and FnBp, which was used to vaccinate mice. There was increased protection in a sepsis model, which was, however, not significantly higher than when vaccination was performed with the two single proteins. Thus, vaccine preparations against single MSCRAMMs or MSCRAMM fusion proteins either had relatively minor success in animal protection models or were not developed further.

MSCRAMM DNA vaccines have been developed with similar preliminary results. An earlier report on a DNA vaccine against ClfA showed that while a strong and specific antibody response could be produced, DNA-immunized mice were not protected against an intraperitoneal challenge with *S. aureus*.[48] In a very recent study, similar results to those for ClfA were obtained with a Cna-DNA vaccine.[49] While a strong antibody and cellular response was produced, no protection was achieved after intraperitoneal infection. Further, ClfA-DNA vaccines produced a strong immune response in cattle.[50] Sera and milk from ClfA-DNA-vaccinated cows were used to preincubate *S. aureus* and reduced *S. aureus* adherence to MAC-T cells. Moreover, they increased neutrophil phagocytosis of *S. aureus*. More recently, Gaudreau et al.[51] immunized mice with a series of plasmids expressing ClfA, FnbpA, and sortase as single proteins or as a fusion protein of all three factors. All vaccines were immunogenic, produced a mixed T_h1 and T_h2 response including functional antibodies (mostly IgG2a), sustained interferon-γ production, and caused a predominantly CD8+ T-cell response. In contrast to the earlier studies with DNA vaccines expressing only one MSCRAMM, multi-gene-vaccinated mice survived in significantly higher numbers when challenged with a virulent *S. aureus* strain; however, there was no significant reduction in clinical signs of arthritis. A combination of MSCRAMM antigens was also used in a DNA vaccine by Castag-

liuolo et al.,[52] in an effort to develop a vaccine for cow mastitis. These authors combined fibrinogen-binding protein Efb, FnbpA, ClfA, and Cna and observed strong antibody and splenocyte proliferative responses. Notably, immunized mice were protected against intramammary challenge with *S. aureus*. These studies demonstrate that the combination of antigens in the case of DNA vaccines resulted in strongly increased vaccine efficacy, in a way similar to the combinatory approaches described in section 8, indicating that DNA vaccine preparations with combined MSCRAMMs should be further studied for use in humans and cattle.

7. Preventing Colonization

The nares are the most important colonization site for *S. aureus* in the human body. Capsule and teichoic acids have been shown to influence nasal colonization.[49,50] Furthermore, it has been demonstrated that there is a correlation between carriage and infection, indicating that infection occurs predominantly when the organism spreads from its primary ecological niches to normally sterile parts of the human body.[13] Prevention of nasal colonization by vaccination could thus theoretically prevent *S. aureus* infection in individuals at risk, and when applied throughout the population, could eliminate *S. aureus* infection on a larger scale. While this approach sounds reasonable and worth pursuing, the epidemiology of CA-MRSA and the routes of transmission of this novel pandemic have not yet been included in the theoretical foundation of preventing *S. aureus* colonization to reduce infection. As far as we know, CA-MRSA infection does not originate exclusively or commonly from nasal or anorectal colonization, but its epidemiology often resembles that of a directly and sometimes sexually transmitted disease.[16,53]

Two studies published at about the same time investigated the prevention of *S. aureus* colonization with anti-MSCRAMM vaccines. Schaffer et al.[54] tested several factors for their influence on nasal colonization in mice. No significant difference compared with the corresponding wild-type strain was detected for the MSCRAMMs ClfA, collagen-binding protein, FnbpA, and FnbpB. Similarly, strains deficient in PIA or the quorum-sensing system *agr* did not exhibit different colonization in this model. This is noteworthy, as *agr* has been an important virulence factor in almost all infection models, and PIA, as a biofilm factor, has been believed to play an important role in colonization. However, experiments were performed with only one *S. aureus* strain for every mutant/wild-type pair. Many different wild-type backgrounds were used and, therefore, it may not be possible to compare the influence of all these factors on nasal colonization with one another. Nevertheless, the authors found that mutants defective in sortase or ClfB showed reduced nasal colonization. On this basis, they vaccinated mice intranasally with a recombinant ClfB vaccine (composed of the A region), with which significant protection from *S. aureus* challenge could be achieved. Interestingly, they also achieved protection from nasal colonization using killed *S. aureus*, which contrasts with the failed attempts to use killed *S. aureus* to prevent infection throughout history. In addition, nasal colonization was reduced by passive immunization with a monoclonal ClfB antibody. Clarke et al.[55] probed bacteriophage expression libraries with sera from infected and uninfected patients to identify immunogenic proteins. They produced 11 of the identified immunogenic determinants as recombinant proteins and measured titers in healthy carriers and noncarriers and in ill individuals. Significantly different titers were detected for 11 antigens comparing ill with healthy individuals and four comparing healthy noncarriers with carriers. Among these four were IsdA and IsdH, and vaccination of cotton rats with either of the two recombinant proteins reduced nasal colonization. Taken together, these findings suggest that, although it is not yet clear whether prevention of colonization will prevent infection, there are data indicating that MSCRAMM vaccines can prevent colonization.

8. A Systematic Approach to Define the Best MSCRAMM Antigen Targets for Prevention of Infection

Unlike many other pathogens, *S. aureus* does not have one defining surface virulence factor. Rather, the basis of pathogenicity in *S. aureus* is multifactorial, and for MSCRAMMs especially there appears to be significant functional redundancy. Thus, more recent efforts have combined different MSCRAMM antigens in vaccines, as outlined in the preceding paragraphs. However, these approaches did not look in a systematic fashion at only a relatively limited number of potential antigens. Another more recent approach by Nabi to increase the efficacy of a next generation StaphVax® conjugate vaccine also represents a largely nonsystematic accumulation of factors believed to be involved in virulence.[56]

In a recently published manuscript, Stranger-Jones et al.[57] took the first broad systematic approach to detect the best antigens among all known *S. aureus* MSCRAMMS. These authors expressed the 19 MSCRAMMs of *S. aureus* identified by genome searches (protein A, FnbpA, FnbpB, ClfA, ClfB, SdrC, SdrD, SdrE, SasA, SasB, SasC, SasD, IsdA/SasE, SasF, SasG/Aap, HarA/IsdH/SasI, IsdB/SasJ, SasK, and IsdC) as His-tagged proteins in *Escherichia coli*, purified them, and injected 100 μg of recombinant protein into mice. Although there were significantly increased IgG titers for almost all antigens, protection from *S.*

aureus challenge varied considerably, as measured by *S. aureus* colony-forming units in the kidneys of infected mice. Reduction of staphylococcal infection was highest with SdrD, SdrE, IsdA, and IsdB as antigens. In comparison, the MSCRAMM ClfA, which had been frequently used for vaccine development in previous studies, had a reduction of about 1 log less compared with the best antigens of that study (SdrE and IsdA), though ranging roughly at the same level as the two other antigens selected by the authors for further characterization (SdrD and IsdB). Reduction of staphylococcal burden by ClfB as antigen was less by 1 log compared with ClfA. FnBpA and FnbpB, other frequently selected antigens in previous studies, were much less efficient in that respect. Opsonophagocytosis of an *S. aureus* protein A-deficient derivative of strain Newman (used to avoid precipitation of antibodies on the bacterial surface due to protein A) was induced by antisera developed against all four antigens. Importantly, opsonophagocytosis was significantly higher with a mixture of antisera developed (singly) against the four antigens, compared with the single-antigen antisera used separately. When a vaccine was produced against a combination of the four antigenic proteins, IgG levels against each of the four antigens were indistinguishable from those achieved by single immunization. However, as the most noticeable result from that study, the combined vaccine provided significant and often complete protection from lethal doses of a variety of *S. aureus* strains in the murine challenge model. With strain Newman, there was significantly increased and, over the time of the experiment, complete protection with the combination vaccine, whereas the single-antigen vaccine preparations did not achieve significant protection. In noteworthy contrast to almost all other studies mentioned in this review, Stranger-Jones et al.[57] tested their combination vaccine with many *S. aureus* strains and based their strain selection on clinical relevance. In addition to strain Newman, they achieved complete protection in their model against challenge with strains NRS248, a strain causing necrotizing pneumonia, and USA400, the prototype CA-MRSA strain, which is, however, now largely replaced by USA300. The latter, which is now by far the most common strain causing skin and soft tissue infections in the US[7] and the almost exclusive source of the current CA-MRSA pandemic, was not included in the study. Results with USA100, the most prominent strain causing hospital-acquired infections in the US, were promising, but no complete protection was achieved. Nevertheless, this study clearly showed that a systematic search for optimal antigens and a combination of those in vaccine preparations yields significantly improved vaccine candidates.

9. Alternatives: Capsule and Others

Alternatives to MSCRAMMs that have been investigated as antigen candidates mainly comprise other, nonprotein surface components. The most prominent examples are the capsule polysaccharides of type 5 and 8, which form the basis of the StaphVax® vaccine developed by Nabi Biopharmaceuticals.[32,33,56] Of the 13 known capsular types in *S. aureus*, types 5 and 8 cause ≈85% of all invasive diseases.[58] Capsular polysaccharides are important for invasiveness and establishment of systemic infections by facilitating resistance to opsonophagocytic clearance.[59] Capsule-type specific antibodies mediate opsonophagocytic killing of the respective *S. aureus* by human neutrophils, and vaccination with capsule polysaccharide conjugate vaccines has provided protection in animal infection models with sublethal and lethal doses.[60,61] However, compared with the capsules of other bacteria that have proved to be excellent vaccine targets (such as those of *Haemophilus influenzae*, *Neisseria meningitidis*, and most notably *Streptococcus pneumoniae*, a frequent colonizer of humans), the role of the *S. aureus* capsule in virulence is minor and less well defined.

Another polysaccharide surface polymer of *S. aureus*, the PIA (or PNAG, poly-N-acetylglucosamine, according to its chemical composition) has long been investigated as a potential vaccine. This polymer is deacetylated in approximately 10–20% of its subunits[22] and deacetylation is important for virulence and immunogenicity.[62,63] Antidiphtheria toxin-conjugated deacetylated PNAG vaccines caused specific opsonic killing of *S. aureus* in rabbits, and passive immunization with the conjugate vaccine protected mice from lethal *S. aureus* challenge.[62] These vaccines may have importance beyond *S. aureus*, as the same polymer is produced by a series of pathogenic bacteria that include coagulase-negative staphylococci (see section 10), *Yersinia pestis*, *E. coli*, and others. In *Y. pestis*, however, the function of PIA/PNAG is mainly to promote biofilm-dependent blockage of the flea midgut and may not be expressed in the human host.[64] Notably, infection of mice with *E. coli* could be prevented by vaccination with a PIA/PNAG vaccine.[65] Finally, PIA/PNAG has also been recognized as an important factor facilitating resistance of *S. epidermidis* to mechanisms of innate host defense, such as secreted antibacterial peptides and non-opsonic neutrophil phagocytosis.[66]

Teichoic acids are sugar alcohol (glycerol or ribitol)-phosphate polymers that are produced by Gram-positive bacteria and have been implicated in nasal colonization and biofilm formation of *S. aureus*.[25,67,68] Although it is not yet clear if those effects are mediated by differential binding of surface proteins,[67] teichoic acids may represent another promising surface-located antigen

candidate. In contrast to many of the other molecules discussed here, a clear advantage of teichoic acids is their ubiquity.

Whether secreted proteins, which contain a series of key virulence factors, may also represent good candidates for vaccine development is largely unknown. Similar to the functional redundancy in the case of MSCRAMMs, no single secreted virulence factors are absolutely required for *S. aureus* virulence in all types of the many manifestations of *S. aureus* disease. One of the most prominent virulence factors appears to be α-toxin, and it might be for that reason that Nabi now proposes to include α-toxin in their new StaphVax® preparation, in addition to teichoic acids and further capsular polysaccharide types.[56] Another antigen to be included in the new StaphVax® is the Panton-Valentine leukocidin (PVL), based on the presence of the *PVL* genes in the CA-MRSA strain USA300.[8] However, it is very unlikely that PVL is a major virulence determinant of CA-MRSA,[63] and whether it is produced *in vivo* at all is unclear.

10. Coagulase-Negative Staphylococci

Coagulase-negative staphylococci, predominantly *S. epidermidis*, are far less virulent than *S. aureus*, but are often implicated in infections of indwelling medical devices.[69] Important in that regard is the exceptional capacity of *S. epidermidis* and other coagulase-negative strain, to form biofilms. *S. epidermidis*, in particular, is a ubiquitous colonizing commensal organism on human mucosal and epithelial surfaces. Thus, it is not entirely clear whether vaccine treatment to eradicate *S. epidermidis* is feasible, as it might significantly disturb the human epithelial and mucosal microflora and possibly enable more harmful microorganisms to take its place. Nevertheless, there might be specific circumstances in which the application of an *S. epidermidis* vaccine may be indicated, such as after surgery and during and following the implantation of medical devices. From an experimental point of view, testing *S. epidermidis* vaccines in animal protection studies is difficult, as immunocompetent mice in particular often do not develop infection at a reasonable rate.

Although not as actively pursued as *S. aureus* vaccines, strategies for vaccine development against *S. epidermidis* are essentially the same as for *S. aureus* and include surface proteins and nonprotein polymers as antigen candidates. In general, we know less about surface proteins of *S. epidermidis*. Using the fibrinogen-binding protein (Fbe) of *S. epidermidis*, Rennermalm et al.[70] achieved reduced severity of systemic *S. epidermidis* infection in mice when bacteria were pre-opsonized with anti-Fbe prior to administration. Sellman et al.[71] (Wyeth Vaccines) have identified immunogenic cell wall-associated proteins of *S. epidermidis* after growth in serum, using 2-dimensional gel electrophoresis and probing with sera from rabbits immunized with live *S. epidermidis*. Twenty-seven open reading frames of proteins with significantly increased serum binding or immunoreactive properties were cloned, expressed as His-tagged recombinant proteins, and used in protection studies. Some vaccines showed promising activity, although statistical significance was not reached. Surprisingly, these included mostly nonsurface proteins, possibly revealing a technical limitation of the study with regard to surface protein preparation.

On the other hand, the presence of genuinely cytoplasmic proteins on the surface of bacteria, due to active export or partial cell lysis, may have yet underestimated implications for antigen selection. Unfortunately, sera from *S. epidermidis*-infected patients were not used in that study, which might have provided more valuable information on possible antigen targets for *S. epidermidis*. Foster's group identified *S. epidermidis* antigenic components expressed during human infection in a way similar to their previous study in *S. aureus*, using a bacteriophage lambda library.[72] The identified proteins were the major autolysin AtlE, which is an abundant noncovalently anchored *S. epidermidis* surface protein, the lipase GehD, and two conserved surface proteins, ScaA and ScaB. While recombinant antigens stimulated an immune response in mice, the study did not investigate whether the vaccines protect from *S. epidermidis* infection.

Nonprotein surface polymers that have been proposed as vaccine candidates for *S. epidermidis* include the aforementioned PIA and PGA. Both polymers have immunoprotective roles for the producing organism, in that they reduce susceptibility to attacks by innate host defense mechanisms.[21,62] While PIA as a structural component of the biofilm matrix is usually secreted in large amounts, PGA production is very low compared with that of *Bacillus* strains that produce PGA. Nevertheless, PGA has a proven function in immune evasion and virulence of *S. epidermidis* and may have exceptional importance as an antigen owing to its ubiquity among *S. epidermidis* strains.

11. Concluding Remarks

We have seen that for several reasons, vaccines against staphylococcal infections are more difficult to develop than for other pathogens. Nevertheless, the severe burden that staphylococcal infections represent to the public health system, now considerably increased by the emergence of the CA-MRSA epidemic, demands renewed efforts in this field. While many researchers are discouraged by the Veronate® and StaphVax® failures and may have turned away from antistaphylococcal vaccine development, the very recent systematic approach to select antigens in a combined vaccine preparation is promising. This type of approach

appears to be more appropriate than the largely nonsystematic combination of virulence factors in a vaccine. In any case, only clinical trials will represent the ultimate test, as is especially important for antistaphylococcal vaccines. Furthermore, the *Staphylococcus* vaccine field would significantly benefit from the inclusion of more clinically relevant strains in early experimental stages, even more so given the changing epidemiology of staphylococcal infections.

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