

Vaccines

The greatest obstacle to development of a vaccine effective against *S. aureus* biofilm infection remains selection of appropriate antigens. The sequencing of the whole genome of multiple *S. aureus* strains,¹²³ followed by those of methicillin-resistant^{124,125} and vancomycin-intermediate¹²⁶ and epidemic¹²⁷ strains undoubtedly marked a significant step forward. The subsequent development of bioinformatics to manipulate and analyze these data has facilitated high-throughput genomic, transcriptomic and proteomic analyses of microbial growth and pathogenesis.^{128,129}

The first question that must be answered is which component of the biofilm should be targeted. Broadly speaking, two alternatives exist: bacterial cells within the biofilm and the biofilm matrix itself. The biofilm matrix may be composed of polysaccharides, protein or extracellular DNA, in proportions that vary between bacterial genera, species and strains. Most anti-biofilm vaccine efforts to-date have been directed toward the extracellular matrix.¹³⁰ Perhaps the best example of this is the staphylococcal polysaccharide inter-cellular adhesion (PIA), or poly-N-acetyl- β -1,6-glucosamine (PNAG), production of which is encoded by the *icaADBC* locus.¹³¹ PIA is produced by both *S. epidermidis*¹³² and *S. aureus*²¹ and is involved in adherence of *S. epidermidis* to host tissues¹³³ and biomaterials.¹³⁴ However, only 57% of *icaADBC*-positive strains¹³⁵ produced a biofilm in vitro,¹³⁶ suggesting distinct strain differences in any correlation of PIA and biofilm formation. The proportion of *ica*-positive *S. aureus* clinical isolates varies according to the clinical origin and even between infection sites that are both biofilm-mediated. For example, the proportion of *icaADBC*-positive *S. aureus* strains was higher in orthopedic prosthesis-associated infections (92%) than in those that were catheter-associated (63%).¹³⁷ There is also some evidence that PIA expression undergoes phase variation.¹³⁸ Although it has been tested against planktonic-type infection in animal models,¹³⁹ the efficacy of vaccination with PIA in preventing biofilm-type infections remains to be determined.

Genomics and its derivatives. Genome-based technologies facilitated rapid identification of vaccine candidates compared with the more conventional approach of identifying and analyzing individual virulence factors from pathogens cultured in vitro.¹⁴⁰ Identification of putative antigens—principally surface proteins—by systematic searching of the pathogen's genome using bioinformatics is termed 'reverse vaccinology'.¹⁴⁰ This has a number of advantages compared with traditional methodologies: (1) there is no need for in vitro culture and (2) antigen selection can proceed independent of in vivo expression levels and/or immunogenicity. As a result, many antigens that would have been passed over in conventional studies may be detected. However, such analyses yield only limited information regarding levels of expression of vaccine candidate antigens during pathogenesis.

Transcriptomic analysis of bacterial pathogens in vivo using microarrays has the potential to generate such data; however, the large quantity of host RNA in any sample makes this problematic. This need for information concerning in vivo expression of potential vaccine antigens led to development of positive- and negative-selection technologies such as recombination-based in

vivo expression technology (RIVET) and signature-tagged mutagenesis.^{141,142} RIVET allows for identification of in vivo expressed antigens during infection whereas signature-tagged mutagenesis identifies antigens required for pathogenesis and survival. These techniques have the advantage of discovering vaccine candidates that in vitro methods may have missed. Additionally, these techniques do not require selective pressure on the bacteria and therefore allow for a natural progression of infection.¹⁴¹

Proteomics and its derivatives. Proteomic profiling identifies the spectrum of proteins expressed by bacteria under varying growth conditions using a combination of two-dimensional gel electrophoresis (2DGE) and mass spectrometry (MS). Detection of membrane and cell wall proteins is a limitation of proteomic profiling due to (1) their relatively low abundance and (2) solubility constraints due to hydrophobicity (the presence of varying numbers of transmembrane domains), and an isoelectric point at pH 8+.¹⁴³ Since vaccine development focuses on surface-associated proteins, use of extraction protocols that solubilize membrane proteins or isoelectric focusing performed in the alkaline pH range are essential. Reference maps of the surface proteomes of *S. aureus* strains Phillips and VISA generated by lysostaphin extraction are available.^{144,145} Another novel antigen discovery strategy involves identification of surface proteins 'shaved' from group A Streptococcus cells by trypsin digestion.^{146,147} Use of this technique has led to discovery of 42 *S. aureus* surface proteins that may have potential as vaccine antigens.¹⁴⁸

Serological probing of proteomic samples, known as immunoproteomics, followed by peptide identification using matrix-assisted laser desorption ionization time-of-flight MS is a direct method for defining antigenic proteins. An initial 2DGE immunoproteomic study of *S. aureus* identified 15 known and novel proteins that were immunoreactive with patient sera.¹⁴⁹ Additionally, subtractive proteome analysis was used to identify immunogenic anchorless *S. aureus* surface proteins. Proteins reacting with an intravenous immunoglobulin (IVIG) preparation (pooled IgG extracted from sera of multiple donors) but not with IVIG depleted of *S. aureus*-specific opsonizing antibodies were considered vaccine candidates.¹⁵⁰ Of a total of ~40 anchorless cell wall proteins identified in this manner, three were cloned. Although not tested in a biofilm model of infection, recombinant versions thereof provided partial protection in a mouse sepsis model.¹⁵⁰ Such anchorless wall proteins lack either a conserved signal peptide or LPXTG motif, and so may have been omitted from classical reverse vaccinology screens.¹⁵¹

An additional consideration when developing vaccines effective against chronic, biofilm-mediated *S. aureus* infection is the physiological heterogeneity of these communities (see Fig. 1). Biofilm communities are inherently complex systems, usually existing in close proximity to a surface. This complexity arises from a number of factors. First, distinct physicochemical gradients are found within microbial biofilm communities.⁴ In most cases, organic compounds, oxygen, or water enter the biofilm from the surrounding bulk fluid and diffuse through the matrix to the depths closer to the surface.¹⁵² Bacteria resident within a biofilm consume these compounds at varying rates, resulting in differential availability of nutrients, dependent on the location

of a particular cell within the community. This effect has been observed experimentally in the case of oxygen tension.¹⁵² The situation is further complicated by very low metabolic levels and radically downregulated rates of cell division of the deeply entrenched microorganisms.¹⁵³

Brady and coworkers used immunoproteomics to elucidate *S. aureus* surface proteins that were immunogenic in the rabbit model¹⁵⁴ of osteomyelitis.⁴⁶ In subsequent work, the same group demonstrated that expression of these antigens during *S. aureus* biofilm growth in vitro was spatially heterogeneous.⁵ Based on these data, four antigens were included in a quadrivalent vaccine administered to rabbits that were then infected with *S. aureus* M2 in a biofilm model of infection of osteomyelitis. Vaccination, in combination with post-infection vancomycin therapy, was effective at preventing development of chronic *S. aureus* osteomyelitis.¹⁵⁵

Despite the considerable human and fiscal resources that have been expended, the search for a vaccine effective against *S. aureus* biofilm infections continues. A caveat to consider in developing anti-*S. aureus* biofilm therapies are the potential losses of treatment efficacy when given to immunocompromised patients at risk of infection, specifically in the case of vaccination. It seems likely that the answer lies in an increased understanding of *S. aureus* biofilm development in vivo, and particularly its interactions with the immune system.

Conclusion

Staphylococcus aureus is a clinically relevant pathogen due to its antimicrobial resistance and evasion of the host immune system.

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In conjunction with the multitude and redundancy of its virulence factors in avoiding host responses and influencing disease, *S. aureus* is able to form intricate micro-colonies termed biofilms. Although neutrophils are capable of invading the biofilm, the bacterial community is able to thwart this attack and may also skew the immune response to survive attack. *Staphylococcus aureus* is the etiological agent to a myriad of human acute infections, however, its ability to form biofilm in host emanates into chronic and recalcitrant disease. Current therapies for treating and preventing chronic biofilm-mediated infections are limited to surgical intervention and prolonged antibiotic regimens or addition of antimicrobial compounds to indwelling-medical devices. Vaccination studies have begun to take biofilm development into consideration, and with the combination of genomic and proteomic-based techniques have identified numerous potential vaccination candidates. However, the physiological heterogeneity and subsequent multifarious protein expression throughout the biofilm must be carefully examined for development of an efficacious *S. aureus* vaccine. Alternatively, modulating the host immune response may prove advantageous in resolving chronic *S. aureus* infections and warrants further investigation.

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Note

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