virulence factors has not yet led to the availability of a vaccine suitable for the human population. Nevertheless, a number of current efforts hold promise for success in the future of *S. aureus* vaccination.

VACCINE STRATEGIES TARGETING *STAPHYLOCOCCUS AUREUS*

Protein Subunit Vaccines

Early investigation into the role of staphylococcal proteins as immunogens was performed in the mid-1900s. Initial attempts involved the production of phage lysates from several *S. aureus* strains. These were evaluated for efficacy in the treatment of human skin infection, demonstrating an 80% recovery rate among the population tested (82). Additional protein-based vaccination strategies include further trials of protein preparations derived from either phage or enzyme-induced lysis of staphylococcal strains (83–85). More recently, whole-killed staphylococci were combined with *S. aureus* toxoids and examined in patients receiving renal replacement therapy via peritoneal dialysis (86). This multicenter, placebo-controlled trial demonstrated an increase in anti-staphylococcal antibodies in the peritoneal dialysis fluid, however, it was unable to demonstrate vaccine efficacy in the protection against peritonitis.

Heralding from the observation that SrtA mutants of *S. aureus* display a virulence defect in animal models of infection (87), a number of groups have examined these proteins as vaccine candidates. Vaccines composed of the individual surface proteins ClfA (88), ClfB (89), IsdB (90), Cna (91), and FnBP (91) have all been demonstrated to confer protection against *S. aureus* challenge. Stranger-Jones et al. used a bioinformatics approach to guide the selection of a group of four SrtA-anchored surface proteins (IsdA, IsdB, SdrD, and SdrE) that were each conserved in eight *S. aureus* genomes (92). Importantly, these surface proteins elicited a host antibody response upon vaccination as independent antigens. Vaccination of mice with each of these antigens in isolation afforded a modest degree of protection from renal abscess formation and mortality following *S. aureus* infection (92). A robust protective response was observed, however, upon the assessment of a vaccine containing a combination of all four surface protein antigens (92). Most importantly, this vaccine was able to confer protection against an array of *S. aureus* clinical isolates, among which are included the LAC/USA300 and MW2/USA400 strains, two extremely virulent CA-MRSA strains that account for a significant proportion of current staphylococcal infections in healthy hosts. Mechanistically, the combined surface protein vaccine yields high-titer antibody responses in the murine host; these antibodies are capable of facilitating neutrophil-mediated phagocytosis of the pathogen.

The most recent approach to the generation of protein-based anti-staphylococcal therapies grew out of the development of a murine model of *S. aureus* pneumonia that facilitated the identification of *S. aureus* α-toxin as a critical virulence factor in pathogenesis (93). This pore-forming cytotoxin exhibits some degree of specificity for erythrocytes and epithelial cells, including the alveolar epithelium that permits gas exchange in the distal lung. *S. aureus* mutant strains devoid of Hla expression were avirulent in the murine model of disease, and similarly, were unable to induce lytic damage to cultured alveolar epithelial cells (93,94). Active immunization with a modified, nontoxic recombinant form of the Hla protein containing a leucine for histidine substitution at residue 35 (H35L) conferred protection against *S. aureus* pneumonia in laboratory animals (95). This protection was also evident upon challenge with the virulent CA-MRSA strains LAC and MW2.

Polysaccharide Vaccines

A single active vaccination protocol targeting *S. aureus* has been evaluated in phase 3 clinical trials. This vaccine, StaphVax (Nabi Biopharmaceuticals, Boca Raton, Florida, U.S.), draws on the successful immunologic approach of coupling a polysaccharide to a proteinaceous carrier. Specifically, StaphVax consists of types 5 and 8 staphylococcal CPs joined covalently to a recombinant form of *Pseudomonas aeruginosa* exotoxin A (rEPA) (96). These conjugates were both immunogenic in mice, generating antibodies that induced opsonophagocytic killing of *S. aureus* by human neutrophils (97). Immunization of healthy volunteers with StaphVax documented a greater than fourfold increase in the CP-specific antibody titers within the immunized population. The serum concentration of these antibodies reached a peak six weeks after immunization, with some decrement in specific antibody titer by six months following immunization (98). The subsequent assessment of this vaccine in phase 2 trials was performed as a multicenter project, investigating the safety and tolerability of the vaccine in end-stage renal disease (ESRD) patients (99). The serum antibody responses to CPs were diminished in these patients, owing to a more rapid decrease in antibody concentration in the serum. The proven safety of this vaccine in the phase 1 and phase 2 trials prompted its examination in a multicenter, randomized double-blind trial. ESRD patients on hemodialysis were enrolled and immunized with StaphVax via a single intramuscular injection (100). The primary end point of the study was protection from invasive *S. aureus* disease in the one-year period immediately following vaccination. Of the 892 immunized individuals, 27 developed *S. aureus* bacteremia in comparison to 37 of 906 controls, a difference that failed to reach statistical significance. Among vaccinated study subjects, more than 80% developed anti-CP antibodies. Interestingly, an assessment of efficacy at the 40-week postimmunization time point did reveal a significant reduction in *S. aureus* bacteremia in the vaccine group relative to the control population. While the study authors observed a correlation between the decline in antibody titers and loss of protection beyond the 40-week time interval, the presence of high-level titers was not necessarily protective in any given individual. StaphVax is currently being reformulated to include not only the types 5 and 8 CP antigens but also CP336 and the two exotoxins, PVL and α-hemolysin.

Studies of the role of PNAG in the pathogenesis of *S. aureus* infection have suggested that this exopolysaccharide may also be a suitable vaccine candidate. Indeed, mice vaccinated with PNAG developed high titers of antibody targeting this antigen, and were protected from *S. aureus* infection in a renal abscess model of disease (65). Together, vaccine studies on the CPs and PNAG illustrate the potentially important role these surface polymers may have in the induction of immunity to *S. aureus*.

Live Vaccination

The origins of live vaccination against *S. aureus* date back to the early 1940s. Mangiaracine and Goodale of the Massachusetts Eye and Ear Infirmary described their preparation of ''young (three- to four-hour)'' cultures of *S. aureus* that were delivered via intramuscular injection according to a fixed five-week protocol to patients suffering from chronic ocular staphylococcal