BETWEEN BEDSIDE AND BENCH

A MRSA-terious enemy among us

Infection with methicillin-resistant *Staphylococcus aureus* (MRSA) can cause symptoms ranging from mild skin infections to more severe disease in various organs, even among healthy individuals. The ability of this pathogen to escape our immune arsenal and overcome antibiotic therapy poses a challenge to preventing their spread and treating the related symptoms. In 'Bench to Bedside', Scott Kobayashi and Frank DeLeo explore new approaches for vaccine development that focus on antigens required for establishment of disease. Studies with infected mice immunized against *S. aureus* coagulases—important for abscess formation and bloodstream infection—suggest such an approach may be used to reduce bacterial load and protect against severe disease in humans. In 'Bedside to Bench', Michael Otto examines a large human study where the presence of genes encoding Panton-Valentine leukocidin toxin (PVL) in community-associated MRSA did not correlate with complicated skin structure infections—a result opposing the widespread notion that PVL is the primary CA-MRSA virulence factor.

■ BEDSIDE TO BENCH

Boosting MRSA vaccines

Scott D Kobayashi & Frank R DeLeo

The pathogen *Staphylococcus aureus*, a leading cause of human bacterial infections worldwide, is capable of causing a diversity of syndromes, from mild and common skin and soft tissue infections to severe, necrotizing and highly invasive disease. The epidemiology of *S. aureus* disease is strongly influenced by rapid acquisition of antibiotic resistance, as some strains become resistant to nearly all front-line antibiotics¹. For example, *S. aureus* acquired resistance to β -lactam antibiotics such as penicillin and methicillin shortly after they were introduced for treatment of human infections¹.

MRSA is a leading cause of hospital-associated bloodstream infections² and the most common cause of community-associated bacterial infections in the US^{1,3}. The outlook for therapeutic options is confounded by a general lack of new classes of antimicrobial agents in the drug discovery pipeline⁴ and the absence of a licensed vaccine for prevention of *S. aureus* disease.

Although there is a clearly defined need for an effective *S. aureus* vaccine, previous efforts to develop a vaccine have been largely unsuccessful^{5,6}. The lack of success is probably owing to the use of conventional vaccine strategies that are directed to enhance opsonophagocytosis, a process in which specific antibodies and serum complement bind bacterial surface antigens and thereby promote ingestion of the microbe by phagocytes (Fig. 1). But this approach is insufficient, as virtually all humans possess antibodies against *S. aureus*, and opsonization

of prominent MRSA strains, such as USA300, with normal human serum promotes efficient uptake by human neutrophils⁷. In addition, there can be considerable survival of ingested *S. aureus* after phagocytosis and consequent destruction of neutrophils⁷.

The forecast for new agents to treat or prevent MRSA has been relatively grim; however, a series of recent studies serves as a springboard for the next generation of *S. aureus* vaccines that do not aim to enhance opsonophagocytic clearance directly^{8–10} (Fig. 1). These studies are based largely on the principle that vaccine antigens should be crucial for establishment of disease. In particular, a recent study by Cheng *et al.*⁹ identified two *S. aureus* blood-clotting factors as essential molecules for the formation of *S. aureus* kidney abscesses in a mouse infection model and showed how immunization against these molecules protects against disease in mice.

S. aureus abscesses are walled-off cavities comprised of fibrin (as a major component of the abscess wall), bacteria, inflammatory cells such as neutrophils (pus) and necrotic tissue. They can form in virtually any tissue of the body, particularly the skin. Abscesses are one of the most common pathological manifestations of *S. aureus* infection and form initially as a host mechanism to prevent the spread of bacteria. But, when left untreated, they can rupture to release bacteria or enlarge to inhibit function of vital organs.

The *S. aureus* clotting factors, or coagulases, known as coagulase (Coa) and von Willebrand factor binding protein (vWbp), activate prothrombin, which then cleaves fibrinogen to fibrin. Cheng *et al.*⁹ found fibrinogen and fibrin distributed diffusely throughout *S. aureus* abscesses in the mouse kidney and more con-

centrated protein levels at the periphery of the lesion. Coa and vWbp also colocalized with prothrombin, fibrinogen and fibrin at specific sites in these abscesses. Using *S. aureus* strains with deletion of the genes encoding Coa and vWbp, both singly and in combination, they evaluated the ability of these strains to cause disease in mouse infections models⁹. A *S. aureus* strain with deletion of both coagulases caused a reduction in lethal bacteremia and fewer kidney abscesses, demonstrating that Coa and vWbp are crucial for pathogenesis.

An early study already suggested that coagulases could have a role in the development of *S. aureus* lesions¹¹. At the time, coagulases were largely considered as nonantigenic and thus not a good target for immune protection against staphylococci. Yet subsequent studies showed that antibodies specific for *S. aureus* coagulase are present in sera from the majority of healthy or infected individuals tested^{12,13}.

Cheng *et al.*⁹ generated rabbit antibodies to neutralize Coa and vWbp function and performed passive immunization experiments in the mouse infection models. These antibodies significantly reduced mortality in mice infected with the community-associated MRSA (CA-MRSA) strain USA300 and largely eliminated staphylococcal abscess formation. Mice vaccinated with purified Coa or vWbp were also protected against formation of USA300 abscesses, suggesting that immunization with these coagulases may be used to protect humans against severe MRSA infections.

Coagulases are attractive candidate vaccine antigens, as most *S. aureus* produce these factors and the mechanism of protection is not based upon generating antibodies that enhance opsonophagocytosis. Rather, the antibodies inhibit coagulase function and the cascade of

Scott D. Kobayashi and Frank R. DeLeo are at the Laboratory of Human Bacterial Pathogenesis, Rocky Mountain Laboratories, US National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA. e-mail: fdeleo@niaid.nih.gov

tive immune responses in the majority of individuals tested with the vaccine and no serious adverse events¹⁶. Another possible maneuver could combine these recently described antigens with other *S. aureus* molecules relevant for infection into a single vaccine formulation—an approach currently under consideration.

The question of whether it is possible to develop an effective vaccine against *S. aureus*, a human commensal microbe, remains open. In an era of high antibiotic use and increasing resistance among bacterial pathogens, it will be necessary to continue moving forward with the development of new vaccine approaches designed to prevent or lessen the severity of MRSA infections.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Figure 1 Vaccine approaches against *S. aureus*, including resistant strains. Left, a vaccine directed to promote or enhance phagocytosis—a traditional bacterial vaccine approach—has the caveat that almost all humans have preexisting antibodies and serum factors that promote efficient phagocytosis of *S. aureus*. Ingested *S. aureus* may also survive after phagocytosis, causing neutrophil lysis. Middle, disease-contributing cytolytic toxins such as α -toxin produced by *S. aureus* can be neutralized with specific antibodies (anti-toxin) to prevent or moderate disease (for example, severe *S. aureus* pneumonia or necrotic skin infections). Right, *S. aureus* secretes coagulases that promote abscess formation and these molecules can be targeted with specific antibodies (anti-coagulase) to inhibit this process.

events leading to accumulation of fibrin and the formation of an abscess wall at the site of infection (Fig. 1).

Previously, this research group used a similar approach to identify additional putative *S. aureus* vaccine antigens, such as the cytolytic toxin α -hemolysin (α -toxin)^{14,15} and IsdA and IsdB¹⁰, which are surface-associated molecules involved in heme uptake by *S. aureus*. Vaccination against α -toxin protected mice against lethal *S. aureus* pneumonia¹⁴ and severe USA300 skin infection¹⁵, and passive immunization with antibodies specific for IsdA and IsdB protected mice against abscess formation and fatal bacteremia by inhibiting heme uptake and utilization¹⁰. A protective mechanism involving vaccine targeting of bacterial heme uptake has the potential to be used broadly for developing new therapies or preventative measures to control antibiotic resistant pathogens.

A second study showed that another protein—*S. aureus* protein A (SpA)—suppresses the humoral immune response, limiting the antibody responses against *S. aureus* and the protection against severe disease⁸. Vaccination of mice with a nonfunctional SpA mutant protein protected mice from MRSA infections by targeting SpA, allowing them to mount normal antibody responses to staphylococcal antigens and preventing or resolving infections more readily.

Collectively, these studies represent a fresh approach to *S. aureus* vaccine design. Inasmuch as these vaccine antigens were effective in preventing or moderating disease in mouse models of *S. aureus* infection, they may be considered further as candidates for a human *S. aureus* vaccine. Indeed, an IsdB-based vaccine known as V710 (Merck) was recently evaluated for safety and immunogenicity in humans in a multicenter phase 1 study¹⁶, showing posi-

■ BENCH TO BEDSIDE

End of the PVL controversy?

Michael Otto

CA-MRSA strains are causing an ongoing epidemic of skin and soft tissue infections, which characteristically develop in healthy individuals without predisposing conditions. The US has experienced the largest CA-MRSA outbreak, with one strain, USA300, being responsible for

the vast majority of infections. In 2005, 13.7% of all invasive MRSA infections in the US were community associated¹. Despite the obvious clinical importance of this disease, the molecular basis underlying the success of CA-MRSA strains as pathogens has remained obscure.

Initial clinical observations had suggested that CA-MRSA virulence is mainly determined by PVL, a bicomponent toxin that is cytolytic toward human neutrophils². Yet, in a recent large-scale epidemiological study published in

Michael Otto is at the US National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.
e-mail: motto@niaid.nih.gov