An antidote for *Staphylococcus aureus* pneumonia?

Frank R. DeLeo and Michael Otto

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of bacterial infections in the United States. Severe invasive MRSA infections, which include pneumonia, are difficult to treat because the bacteria are resistant to antibiotics. A new report now shows that immunization against α-hemolysin (Hla), a cytolytic toxin secreted by most *S. aureus* strains, protects mice against lethal pneumonia. This finding represents the first successful vaccine strategy for the treatment of staphylococcal pneumonia.

S. aureus is a leading cause of bloodstream, skin, soft tissue, and lower respiratory tract infections worldwide (1). In developed countries such as the United States, resistance to β-lactam antibiotics in MRSA is a major problem in hospitals and other healthcare settings. In these settings, *S. aureus* infections are primarily caused by MRSA and typically occur in individuals with risk factors for disease, such as those who are immunocompromised or have had surgery. Notably, the incidence rate of all invasive MRSA infections, including those outside of hospitals, is high compared with other bacterial pathogens (31.3 per 100,000 individuals), and 20% of these infections result in death (2). Although bacteremia is the most prevalent condition during invasive disease caused by MRSA, pneumonia ranks second and occurs in ∼13.3% of all invasive infections (2).

In contrast to *S. aureus* infections acquired in healthcare settings, community-associated *S. aureus* infections, which in the United States are also caused primarily by MRSA, occur in otherwise healthy individuals. The majority of community-associated MRSA (CA-MRSA) infections are treatable infections of skin and soft tissue (3), but some infections lead to severe invasive disease (4). CA-MRSA was first reported in the late 1990s, when pneumonia was the third most prevalent syndrome, occurring in 13.5% of infected children (5). The most prevalent CA-MRSA isolate, known as USA300, accounts for up to 97% of all CA-MRSA infections (6).

Past efforts to generate an effective vaccine against *S. aureus* have thus far been unsuccessful. A new report by Wardenburg and Schneewind on page 287 in this issue (7) shows that immunization with the *S. aureus* virulence factor Hla protects mice from an otherwise lethal *S. aureus* infection.

Virulence factors and immune evasion

S. aureus encodes a remarkable repertoire of virulence factors. These molecules promote host colonization, facilitate evasion of the human innate immune system, and alter immune responses (for review see reference 8). For the purposes of this commentary, we will limit our discussion to a few *S. aureus* surface molecules, some of which have been used previously as vaccine targets.

Human neutrophils are a primary cellular defense against bacterial infections. Previous studies have shown that host opsonins, such as serum complement and antibody, play a major role in the phagocytosis of *S. aureus* by neutrophils (9–11). *S. aureus* makes several proteins, including protein A, serotype 5 or 8 capsular polysaccharide (CP5 or CP8), and clumping factor A (ClfA), which inhibit phagocytosis (12–14). But despite the bacteria's efforts to evade neutrophils, normal human serum contains a sufficient number of opsonins to promote their rapid uptake by these cells (15). The majority of clinical isolates, including USA300, encode ClfA and CP5 or CP8 (14). Because antibodies specific for CP5 or CP8 enhance phagocytosis, CP5 and CP8 have been evaluated extensively as vaccine antigens (16–18). In the end, however, *S. aureus* vaccines designed to enhance bacterial uptake have had limited success.

One possible reason for this outcome is the lack of correlation between uptake of the bacteria by neutrophils and their subsequent destruction. For instance, the most prominent CA-MRSA isolates survive relatively well inside neutrophils, probably in part because of their ability to resist the effects of neutrophil-derived reactive oxygen species and antimicrobial peptides (19, 20). The neutrophils, on the other hand, undergo rapid lysis after uptake of these strains (Fig. 1) (15, 21). The ability of *S. aureus* to survive after phagocytosis has lead some to suggest that neutrophils could be a vector for disseminating bacteria (22, 23). *S. aureus* can also persist inside macrophages for several days, ultimately causing the death of the cells in a process that depends on Hla (24).

Because uptake does not necessarily correlate with the killing of *S. aureus*, high titers of anticapsule antibodies, which facilitate uptake, may not protect against disease. This notion is not new, as it has long been known that virtually all humans have circulating antistaphylococcal antibody, and yet some still become infected (25). The idea that antibodies against the bacterial capsule may not provide protection was borne out in two unsuccessful phase III clinical trials designed to test the efficacy of active immunization against the *S. aureus* antigens CP5, CP8, and ClfA (26).

Hla, a pore-forming cytolytic toxin that assembles as a heptameric β-barrel structure in the plasma membrane of susceptible cells, is arguably the most widely studied *S. aureus* toxin (for review see reference 27). The toxin is known to cause destruction of a wide-range of host cells, including erythrocytes,

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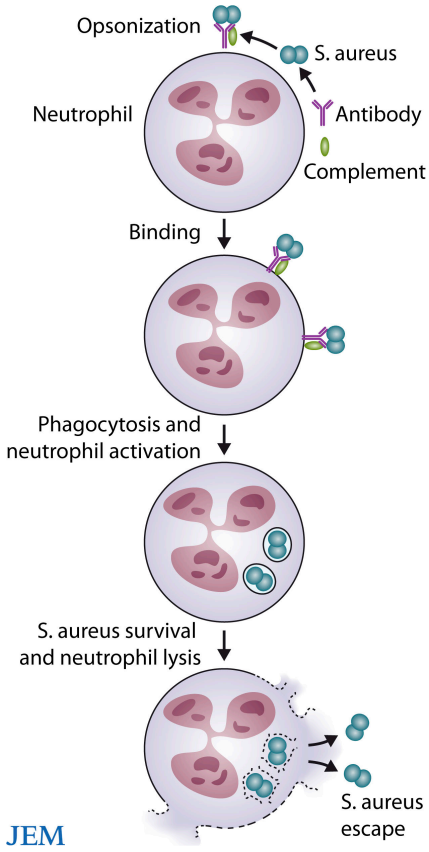

Figure 1. Potential pitfall of *S. aureus* vaccines designed to enhance bacterial uptake by neutrophils. *S. aureus* is opsonized by bacteria-specific antibody and serum complement, which promote rapid binding and uptake of the bacteria by neutrophils. After uptake, *S. aureus* uses multiple mechanisms to survive and cause the death of the cell, allowing the escape of sequestered bacteria

epithelial cells, fibroblasts, and monocytes. Hla gained notoriety in 1928 when it was implicated in the deaths of 12 Australian children who had received a diphtheria toxoid vaccine that was later found to be contaminated with an Hla-producing *S. aureus* strain (27). Although anti-Hla antibody therapy was studied intensively, interest in this approach waned during the antibiotic era. *S. aureus* encodes numerous other extracellular cytolytic toxins, including δ-hemolysin, γ-hemolysin, Panton-Valentine leukocidin (PVL), leukocidin D/E, a leukocidin homologue (LukM/F′-PV), and the newly described phenol-soluble modulin-like peptides (28). The relative contribution of Hla to human disease as compared with these other virulence factors is not known, in part because susceptibility to Hla varies among different animal species.

A vaccine approach for treatment of *S. aureus* pneumonia

Until now, vaccination against Hla has not been tested in an *S. aureus* pneumonia model. In this issue, Wardenburg and Schneewind show that immunization against Hla prevents *S. aureus* pneumonia (7). The authors first show that the severity of lung disease in mice correlates with the levels of Hla produced by a particular *S. aureus* isolate (7). These findings are consistent with a recent study from the same group demonstrating that Hla is important for the pathogenesis of CA-MRSA pneumonia (29).

In the new study, mice were immunized with a nonpore-forming Hla variant, Hla_{H135L}, and challenged intranasally 3 wk later, a protocol that typically induces lethal pneumonia (29). Immunization with Hla_{H135L} protected 90–100% mice against all *S. aureus* strains tested (7). Vaccine-induced protection correlated directly with reduced inflammation and less severe destruction of lung tissue. Passive immunization with Hla antibody 24 h before intranasal challenge with *S. aureus* also protected animals against an otherwise lethal intranasal challenge with CA-MRSA or an antibiotic-sensitive *S. aureus* strain (7).

Antibodies against Hla also protected human lung epithelial cells from *S. aureus*–induced lysis (7). Although these results indicate that Hla contributes to lung tissue destruction, it is not yet clear whether the animals' death resulted from direct destruction of lung cells by the toxin, from an excessive inflammatory response, or from both. Passive transfer of Hla antibodies significantly reduced circulating levels of interleukin 1β, a cytokine known to accompany acute lung injury. Therefore, it is reasonable to conclude that the inflammatory response may contribute to Hla-mediated lung damage (Fig. 2).

A role for other toxins?

There has been considerable debate about whether another *S. aureus* toxin, PVL, is essential for CA-MRSA virulence. In their report, Wardenburg and Schneewind found that, unlike antibodies specific for Hla, antibodies specific for PVL did not protect mice against *S. aureus* pneumonia (7). This finding was wconsistent with an earlier study by this group, which also suggested that PVL is not required for CA-MRSA–induced pneumonia in mice (29).

This result appears to conflict with earlier data suggesting an essential role for PVL (30). But that study relied on laboratory strains of *S. aureus* that overexpressed PVL, thus clouding the physiological significance of these findings. Indeed, studies conducted using animal models of CA-MRSA disease have unanimously suggested that PVL is dispensable for bacterial virulence (21, 29). Recent work has, however, highlighted the potential importance of other virulence factors in CA-MRSA disease (28, 29).

Bacterial toxins as vaccine targets

There are numerous examples of successful vaccination against bacterial toxins,

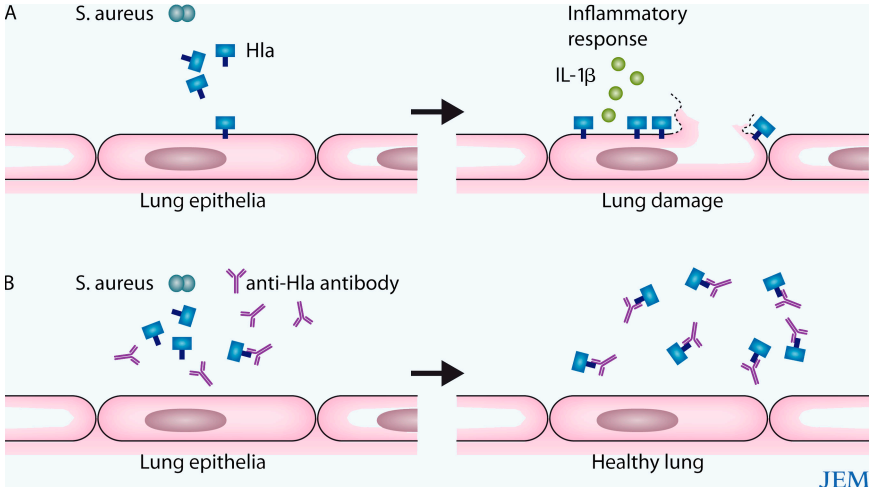

Figure 2. Hla-induced pneumonia and mechanism of protection by Hla antibodies. (A) Hla may cause direct destruction of lung epithelial cells and/or elicit an inflammatory response, including production of proinflammatory cytokines such as interleukin 1β, which contributes to pneumonia. (B) Anti-Hla antibody blocks Hla, thus inhibiting its cytotoxic effects.

including botulinum, diphtheria, and tetanus toxins. However, these toxins are known to be the primary causative agents of disease induced by their respective organisms. In contrast, *S. aureus* produces many toxins, and it has been generally accepted that no single *S. aureus* extracellular molecule can trigger disease on its own. This idea is called into question by the finding that Hla alone is required for *S. aureus* pneumonia (7, 29). The high level of infections caused by the *S. aureus* isolate USA300 and the abundance of Hla produced by this strain in vitro suggest that targeting Hla during invasive CA-MRSA infections may be a promising therapeutic approach.

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