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Summary

The recent spread of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) has brought increasing concerns of heightened disease severity and persistence following invasive disease. In line with the need for new treatment paradigms, two recent reports have shown that antibody-based therapies can restrict acute *S. aureus* infection and persistence, and improve pathological symptoms.

Despite its ubiquitous and mostly benign presence as part of human flora, *Staphylococcus aureus* is responsible for a large variety of diseases in both community and hospital settings (Lowy, 1998). The past decade has witnessed an alarming expansion in the burden of invasive staphylococcal disease, particularly disease caused by methicillin-resistant *S. aureus* (MRSA) strains, resulting in severely restricted antimicrobial treatment options. The emergence of community-associated MRSA (CA-MRSA) strains accounts for much of this burden. The propensity of CA-MRSA to cause a severe toxin-mediated necrotizing pneumonia after influenza-like illness suggests an ominous linkage between these difficult-to-treat strains and an imminent public health threat. Patients with invasive disease are in danger not only of bacteremia and sepsis, but also of metastatic infections that frequently persist and relapse despite optimal treatment. The two polar forms of *S. aureus* disease, acute and persistent, have clinical, microbiological, and immunological distinctions. Thus, to manage *S. aureus* we need new treatment paradigms that are syndrome-specific.

Antibodies, the products of B cells, have diverse functions in physiopathologies, including infection. They can neutralize pathogens and toxins and also reduce host-damage associated with infection. In addition, antibodies are versatile therapeutic tools that can be improved structurally or by drug conjugation, a property used in cancer therapy. Recently, two studies (Diep et al., 2016; Lehar et al., 2015) explored these properties of antibodies to target MRSA in experimental models.

Although *S. aureus* is an extracellular bacterium, it can adapt an evasive mechanism by entering host cells and phagocytes where it can better survive assault by synthetic and host antimicrobials and antibodies. Lehar et al. found that intracellular MRSA are not only protected from currently used antibiotics but also serve as seed for dissemination of infection to other tissues (Lehar et al., 2015). To overcome the inability of antibodies and antibiotics to eradicate intracellular bacteria, the authors designed a novel antibody-antibiotic conjugate (AAC) intended to be activated specifically inside host cells that have taken up *S. aureus*.

AAC consists of an anti-*S. aureus* antibody (THIOMAB) that recognizes pathogen-specific cell wall teichoic acid (WTA) and is covalently linked to the rifamycin derivative rifalogue by a cathepsin-cleavable linker. The antibody recognizes cell-free *S. aureus*, leading to opsonization. The AAC-bacterial complex is taken-up by phagocytic cells via Fcγ-receptors, and once inside the phagolysosome, rifalogue is released from AAC through cleavage of the linker by cathepsins. Rifalogue then kills the bacteria intracellularly (Figure 1). Rifalogue was chosen because it retains bactericidal activity in the acidic environment of the phagolysosome and because it can kill non-replicating bacteria and antimicrobial-tolerant persister cells. AAC was inactive outside host cells, confirming the safety of the construct.

In vitro experiments demonstrated that AAC could not only eradicate intracellular *S. aureus,* but it also significantly reduced cell-to-cell transfer of bacteria. *In vivo* experiments in mice demonstrated superiority of AAC over vancomycin in the treatment of bacteremia. Although prophylaxis with vancomycin or treatment immediately following infection with MRSA eliminated the bacteria, the efficacy of vancomycin was greatly diminished in mice having established infection (Lehar et al., 2015). Extracellular *S. aureus* can quickly sequester within phagocytes, potentially explaining the inability of antibiotics to clear infection once it is established when compared to AAC that targets MRSA's intracellular niche.

Lehar et al. also made an interesting observation about antibody therapy for MRSA. *S. aureus* is a commensal bacterium, and most humans have *S. aureus*-specific antibodies. Indeed, Lehar et al. found that human serum/plasma contains nearly 300 μg/ml anti-*S. aureus* antibodies of which 1/3 are directed against WTA antigens (Lehar et al., 2015). Likewise, therapeutic intravenous immunoglobulin G (IVIG), obtained from the pooled plasma of several thousand healthy donors, contained similar amounts of anti-*S. aureus* antibodies. IVIG is mainly used in the therapy of primary immunodeficiency, autoimmune, and inflammatory conditions (Gilardin et al., 2015). Because of the presence of antibodies to various pathogens and pathogen-derived molecules, IVIG confers protection against infections when used in immunodeficient patients. Consequently, IVIG therapy has been used for several bacterial and viral infections. However, prophylaxis of mice with either MRSA-specific unconjugated anti-WTA antibodies or IVIG failed to prevent kidney infection after intravenous injection. These data suggested that antibody-mediated opsonization fails to eliminate MRSA and agree with clinical studies indicating that anti-staphylococcal immunoglobulins targeting surface motifs fail to prevent staphylococcal infection in low birth weight infants (Shah and Kaufman, 2009).

IVIG contains antibodies to various toxins including Panton-Valentine leukocidin (PVL), a cytotoxin associated with CA-MRSA, necrotizing pneumonia, and skin and soft-tissue infections (Gauduchon et al., 2004). Several case reports support use of IVIG for necrotizing pneumonia caused by MRSA, in analogy to its beneficial effects in toxin-mediated streptococcal infection. Based on these data and proven anti-inflammatory mechanism of IVIG, Diep and colleagues explored IVIG therapy in a rabbit model of necrotizing pneumonia caused by CA-MRSA and hospital-associated MRSA (HA-MRSA) (Diep et al., 2016).

Diep et al. found that prophylaxis or early treatment with low-dose IVIG (200 mg/kg), either alone or in combination with antibiotics, significantly improved survival of rabbits infected with a CA-MRSA or HA-MRSA strain (Diep et al., 2016). Maximal efficacy was observed when IVIG was combined with antibiotics. Analysis of deletion mutants reveled that α-hemolysin (*hla*) and *pvl*-toxin genes contributed to pathogenesis of necrotizing pneumonia. Interestingly, anti-Hla and anti-PVL IgG, affinity purified from IVIG, were protective when used prophylactically whereas the depleted IVIG fraction failed to protect the animals (Figure 1) (Diep et al., 2016). These data suggest that antibody–based therapies should target secreted toxins rather than surface proteins to alleviate the pathology of necrotizing *S. aureus* pneumonia.

Although IVIG improved the survival of animals, it did not eradicate bacteria at infecting sites, consistent with the results of Lehar et al. It is also not known whether IVIG can protect animals after established infection, where bacterial load and toxin levels could be high. Nevertheless, these data suggest that IVIG should be explored in the therapy of necrotizing pneumonia caused by *S. aureus*. Since immunoglobulin classes differ in function, comparison of IVIG efficacy with that of IgM-enriched immunoglobulin preparations and pooled normal IgA preparations is also needed.

Ideally, antibody-based therapy aimed at necrotizing pneumonia should target bacteria as well as toxins and bacterial products. In this scenario, IVIG would protect the host from toxins and overwhelming sepsis, while AAC therapy would eliminate the intracellular reservoir of bacteria associated with bacteremia and metastatic infection. Although, IVIG contains antibodies to toxins of MRSA, antibodies usually have low-to-medium affinity. Therefore, IVIG could be supplemented with monoclonal antibodies (MAb) directed at specific toxins to enhance the efficacy of toxin neutralization while retaining the anti-inflammatory properties of IVIG. In support of this strategy, antibodies to PVL (Laventie et al., 2011), α-hemolysin (Hilliard et al., 2015) and staphylococcal enterotoxin B (SEB) (Varshney et al., 2014), a potent superantigen, have shown benefit in various animal models of *S. aureus* infection. Notably, the data from Diep et al. did not indicate that protection rendered by IVIG was associated with its anti-inflammatory mechanisms. However, the anti-inflammatory effects of IVIG are usually observed with higher doses that are used in clinical settings of autoimmune and inflammatory conditions, suggesting that anti-inflammatory effects may still impact clinical efficacy.

Three additional comments are relevant to the reports described above. First, patients in whom adjunctive treatment is likely to be effective may not be identifiable early in the course of infection, prior to sepsis or disseminated infection, when therapy is most beneficial.

Second, given that CA-MRSA strains cause strikingly severe infections in community subjects, their potential contribution to severe nosocomial infections has attracted attention. However, Diep and colleagues point out recent work suggesting that attempts to draw direct relationships between acute virulence attributes, strain success, and patient outcomes may have been too simplistic (Rose et al., 2015). Adaptation to the hospital environment and poor clinical outcome are often associated with low — not high — virulence. Thus, translation of these therapeutic strategies to nosocomial necrotizing pneumonia is not straightforward.

Third, unlike experimental animals, humans have readily detectable *S. aureus*-specific antibody responses. Also, by binding to the Fcγ portion of IgG, staphylococcal protein A coats *S. aureus* with non-specific antibodies, reducing the binding of high-affinity antibodies and opsonization. This issue should not pose a problem for IVIG or MAbs that target secreted toxins. However, although AAC therapy was effective even in the presence of competing antibodies to surface motifs of *S. aureus*, only clinical trials can determine whether AAC can opsonize non-specific IgG-coated *S. aureus* and eliminate bacteria in patients.

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FIGURE LEGEND

Figure 1. Tackling difficult *Staphylococcus aureus* infections by antibody-based therapies.
(A) Elimination of the intracellular pool of *S. aureus* during bacteremia using an antibody-antibiotic conjugate (AAC). The *S. aureus* wall teichoic acid (WTA)-specific IgG of AAC opsonize free bacteria and are taken-up by phagocytic cells via Fcγ-receptors. Inside the phagolysosome, antibiotic (rifalogue) is released from AAC through cleavage of the linker by cathepsins. Rifalogue then kills the bacteria.
(B) IVIG therapy protects rabbits from necrotizing pneumonia caused by MRSA. The protection by IVIG is mediated mainly via IgG to critical toxins Panton-Valentine leukocidin (PVL) and α-hemolysin (Hla) that contribute to the pathogenesis of necrotizing pneumonia.