A new approach to toxin neutralization in *Staphylococcus aureus* therapy

Dane Parker & Alice Prince

***Staphylococcus aureus* is a significant human pathogen responsible for a range of diseases including pneumonia, sepsis, skin, and soft tissue infections. An important component of its success as a human pathogen is the production of a large array of virulence factors including several toxins. In this issue of EMBO Reports, Reyes-Robles and colleagues [1] identify a glycine-rich motif shared by bicomponent leukocidins. When this motif is deleted, the altered toxin exerts dominant-negative effects that neutralize leukocidin function and thus represents a potentially novel avenue for *S. aureus* therapy.**

See also: T Reyes-Robles *et al* (March 2016)

Effective strategies to treat or prevent staphylococcal infection have been limited by the ability of these organisms to adapt to a variety of clinical settings. Such adaptation includes changes in metabolic activity and in the expression of virulence factors and toxins. Depending on the site of infection, *Staphylococcus aureus* proliferates using aerobic metabolism or anaerobic pathways at oxygen-depleted sites [2]. It adapts to aerobic glycolysis at many sites including skin and immune cells [3]. The importance of neutrophil function in the effective eradication of *S. aureus* at all sites of infection and under these different metabolic conditions has been well documented. These organisms, nonetheless, are highly resistant to phagocytic clearance and express multiple gene products that directly thwart immune function [4].

In contrast to many human pathogens that evade proinflammatory signaling to survive, *S. aureus* is unusually resistant to phagocytic clearance and persists despite the activation of a robust proinflammatory

response [4]. Staphylococcal proteins block neutrophil chemotaxis (CHIPS), interfere with opsonization (protein A and Sbi) and complement binding (Cna). *Staphylococcus aureus* surface proteins interfere with neutrophil mobilization and activation, while others inhibit neutrophil serine proteases. Once within the phagocyte, *S. aureus* gene products contribute to escape from within the phagosome and activate inflammasome signaling, which contributes to neutrophil recruitment and tissue damage.

Once leukocytes have been recruited to the site of staphylococcal infection, the organisms express multiple toxins, many of which are specific for human leukocytes. These leukocidins are commonly expressed in clinical isolates of *S. aureus* and lyse human neutrophils, which are readily replenished from bone marrow stores. Specific toxins deplete immunomodulatory macrophages that are not as readily replaced. The five bicomponent leukocidins of *S. aureus* are composed of two heterologous subunits that form β -barrel pores leading to host cell lysis [5]. These include Pantan-Valentine leukocidin (PVL, LukSF), leukocidin AB (LukAB), leukocidin ED (LukED), and gamma hemolysin (two toxins HlgAB and HlgCB) [5]. Although the bicomponent toxins share substantial homologies, each has distinct receptors. LukED targets cells with the CCR5, CXCR1, and CXCR2, while HlgAB targets CCR2, CXCR1, and CXCR2. The toxins LukSF, LukAB, and HlgCB all are highly specific for human receptors. LukSF and HlgCB target C5aR and C5L2, whereas LukAB binds CD11b [5].

In this issue of *EMBO Reports*, Reyes-Robles and colleagues identify a glycine-rich motif in the stem domain of these toxins that penetrates target cells and causes neutrophil lysis [1]. Toxins lacking the glycine-rich

motif do not cause cytotoxicity and exert a dominant-negative effect in the presence of the same or a heterologous bicomponent toxin. This is due to the formation of mixed complexes that block the receptor and result in defective pore formation (see Fig 1). The modified toxins prevent toxicity caused by native protein, by toxin in WT culture supernatant, or by live *S. aureus*. Specific dominant-negative toxins have differing levels of cross-reactivity that are receptor specific. The mutant HlgCB toxin affords better protection to HlgCB and LukSF (both of which target C5aR and C5L2), whereas mutant LukED protects against LukED itself and HlgAB, which target CXCR1. Importantly, the dominant-negative forms of the toxin protect mice from lethal challenge with purified LukED or HlgAB. The mutant LukED is protective *in vivo*, reducing bacterial burden in the liver of *S. aureus*-challenged mice. These experiments suggest that such dominant-negative toxoids or inhibitors to this glycine-rich motif could provide a therapeutic adjunct to prevent the cytotoxicity of *S. aureus*.

This study also raises many additional questions. The abundance of the receptors for the different bicomponent toxins on immune cells, besides neutrophils, will influence the efficacy of the dominant-negative toxoid approach. As the cross-reactivity of the mutant toxins is receptor dependent, knowing which immune cells are likely to be protected will be important in designing a cocktail of therapeutic toxoids. Determining the relative prevalence of the bicomponent toxins expressed in clinical isolates will also be important and their relative expression at different sites of infection. Since LukAB shares significantly less sequence conservation, targeting this toxin may not provide cross-protection and may need to be targeted

Figure 1. Dominant-negative toxin subunits block pore formation.

Wild-type *Staphylococcus aureus* produces the bicomponent toxins as two subunits (white and purple) that bind to their receptor, oligomerize, and create a pore in the membrane leading to cell lysis. When one of these subunits is mutated and lacks the glycine motif, it becomes dominant negative (red). The dominant-negative subunit associates with native subunits, binding to the receptor and oligomerizing, but pore formation does not occur, inhibiting cell lysis.

individually if it is important in pathogenesis. Assessing therapeutic utility with a combination of different dominant-negative toxoids may be challenging, as efficacy is not accurately reflected in the animal models that lack the relevant receptors.

The use of a peptide antagonist or dominant-negative protein to ameliorate *S. aureus* infection represents a departure from existing preclinical developments. Given the difficulties in anti-staphylococcal vaccine development, and the clinical failures of antimicrobial therapy even against antibiotic susceptible strains, new approaches to treat staphylococcal infection are needed. The potential to target multiple toxins is clearly important, given the tremendous repertoire of toxin production by staphylococci. However, there are pharmacological challenges in the development of therapies based on protein delivery. Protein stability is an important issue raised also by the authors, as treatment with the dominant-negative protein 24 h prior to infection did not result in protection. Antigenicity will also be a likely problem. A significant amount of work in *S. aureus* therapy has been focused on monoclonal antibody therapy. The α -toxin is a major virulence factor [6] that targets epithelial cells, platelets, and immune cells. An anti- α -toxin antibody is being commercially developed, now in phase 2b testing, that is effective in preventing morbidity and mortality in mouse

models of pneumonia [7] and skin infection [8]. Mice treated with this anti- α -toxin antibody have significantly reduced inflammation, proinflammatory cytokine production, and cell damage within the constraints of the model systems. A more broadly specific monoclonal Ab that can recognize α -toxin as well as HlgAB, HlgCB, LukED, and LukSF is also under development, leaving LukAB as the only bicomponent toxin not neutralized [9]. Recent research has demonstrated the effectiveness of an antibody–antibiotic conjugate to eliminate intracellular *S. aureus* [10]. This conjugate consists of an antibody directed toward *S. aureus* wall teichoic acid conjugated to a rifampicin derivative that is activated once proteolytically released in the phagolysosome.

The prospect of therapeutic agents that directly target *S. aureus* virulence factors, preventing cytotoxicity and loss of immune cell function, would be a highly desirable addition to the current, often inefficient therapy given to patients with *S. aureus* infection. Several challenges remain: the ability to develop a toxoid or antagonist that can target all the bicomponent toxins, documentation of effectiveness in an *in vivo* model, and how the dominant-negative constructs might function in the presence of antimicrobial agents. It will be important to establish whether their pharmacokinetic properties would permit use in prophylaxis and

perhaps, most importantly, whether widespread use of such toxoids would ultimately result in the selection of mutants resistant to the dominant-negative effect. Nonetheless, the identification and recognition of the utility of a common glycine-rich motif provides the opportunity for the development of novel anti-staphylococcal therapeutics.

References

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