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Cytolysins, Superantigens, and Penumonia due to Community-Associated Methicillin-Resistant *Staphylococcus aureus*

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Abstract

This editorial evaluates data in an accompanying manuscript by Hongo et al. and includes comparison to other published data on virulence factors (cytolysins and superantigens) associated with or contributing to severe pulmonary diseases caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The major conclusion of the Hongo et al. article is that studies to assess cytolysin functions in serious human MRSA infections must use non-murine models, since cytolysins, such as Panton-Valentine leukocidin (PVL), have limited activity in killing mouse polymorphonuclear leukocytes (PMNs), compared to killing human PMNs.

In a 2007 collaborative study analyzing 2005 data, the Centers for Disease Control and Prevention (CDC) reported that *S. aureus* is the most significant cause of serious and fatal infections in the United States [1]. MRSA strains are well-recognized as significant infection problems for hospitals. In the last 15 years, community-associated MRSA (CA-MRSA) have emerged to become highly significant causes of skin and soft tissue infections, and highly fatal septicemia and pulmonary diseases [2–4]. In 1999, four children were reported who had pulmonary, community-associated MRSA identified as CDC USA400 by pulsed-field gel electrophoresis, and all four children succumbed [2]. Their MRSA isolates have the following secreted virulence factor phenotype: cytolysins α-toxin^{+}, γ-toxin^{+}, PVL^{+}, and phenol-soluble modulins (PSMs)$^{+}$; and superantigens staphylococcal enterotoxin (SE) B^{+} or C^{+}. In 2003, CA-MRSA CDC USA300 isolates emerged, also associated with skin and soft tissue infections, and life-threatening septicemia and pulmonary diseases [3]. Their secreted virulence factor phenotype includes: cytolysins α-toxin^{+}, γ-toxin^{+}, PVL^{+}, and PSMs^{+}; and superantigen staphylococcal enterotoxin-like (SE-l) Q^{+}. A recent study suggests that some of these USA300 isolates also make a deletion mutant form of toxic shock syndrome toxin-1 (TSST-1) [5]. Although not generally recognized, CA-MRSA CDC USA100 and 200 isolates are emerging. These isolates have multiple secreted virulence factor phenotypes, including combinations of: cytolysins α-toxin$^{+/-}$, γ-toxin$^{+/-}$, PVL$^{+/-}$, and PSMs^{+}; and superantigen TSST-1^{+} (a significant emergent strain within this category has the phenotype cytolysins α-toxin^{-}, γ-toxin^{-}, PVL^{-}, and PSMs^{+}; and superantigen TSST-1^{+}). It is important to remember that methicillin-sensitive versions (MSSA) of these same PFGE type organisms exist, and these MSSA the same types of infections. With emergence of severe pulmonary diseases caused by CA-MRSA, investigators have assessed the roles of secreted virulence factors. These diseases most often occur secondary to viral infections and in association with pre-existing conditions such as asthma; they represent highly important fractions of CA-MRSA infections. The pulmonary diseases most recognized include necrotizing pneumonia [2,3], purpura fulminans [6,7], and post-viral toxic shock syndrome (TSS) [8].

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The most studied virulence factors in serious pulmonary infections have been the cytolysins, including the accompanying Hongo et al. manuscript. Much of the research has focused initially on CDC USA 300 and USA400 strains. When initially described, these isolates associated with necrotizing penumonia, were reported to secrete PVL [9,10]. Numerous clinical studies quickly emphasized the high association of PVL with illness. There followed multiple studies to determine if PVL is a significant virulence factor in causation of necrotizing pneumonia or is simply a "tag-along" marker for CA-MRSA strains. The first of these studies reported by the DeLeo research group, analyzed disease production in mice with use of isogenic CA-MRSA strains, differing only in PVL production [11]. Their study demonstrated that PVL is not critical in disease production, since both PVL^+ and PVL^- strains cause comparable illness. The authors also showed that PVL^+ and PVL^- strains lysed human PMNs comparably. Their paper was followed by a collaborative study, authored by Labandeira et al. [12], in mice that showed PVL is critical to disease production, creating a significant controversy. More recently, the DeLeo group teamed up with Otto and Schneewind research groups and examined the role of other cytolysins in serious pulmonary diseases [13–15]. These studies in mice convincingly demonstrate that both α-toxin and PSMs are key participants in serious CA-MRSA illnesses, but not PVL. Finally, the Hongo et al. study has evaluated the potential roles of both PVL and PSMs in serious diseases by studies of cytolysin effects on mouse and human PMNs. Their studies demonstrate that mouse PMNs are resistant to PVL, whereas human PMNs are susceptible. Additionally, their studies show that PSMs as produced by CA-MRSA are not key cytolysins for human PMNs, but PSMs can augment PVL lytic activity.

My analysis of these studies is summarized as follows:

1. The major role of PVL in necrotizing pneumonia is hypothesized to be cytotoxicity for human PMNs and possibly other pulmonary cell types. Clearly PVL is cytotoxic for human PMNs, but results from the above studies in mice are disparate. It is unclear how PVL can lack importance in serious disease in mice in one study [11], and yet be critical in a later study [12]. The studies of Hongo et al. do not resolve this controversy, but appear to support the finding of Labandeira et al. [12]. PVL is a hetero-chain, heptamer pore-forming cytotoxin that belongs to the larger γ-toxin cytolysin family [16]. Similarly, α-toxin is well-established as a potent human cell cytotoxin; this toxin is a homo-chain, heptamer pore-forming toxin [17]. All of these cytolysins (PVL, α-toxin, and γ-toxin [not evaluated in any of the above studies]) have cytotoxic activities for humans cells, including PMNs and epithelial cells, form pores of the same approximate size, but differ somewhat in cytotoxic potency dependent on host cell type. The cytolysins are redundantly produced by CA-MRSA, and it is reasonable to suggest that each cytolysin participates in necrotizing pneumonia in humans in accordance with both amounts produced and potency. In our hands, both α-toxin and PVL have cytolytic and pro-inflammatory activity for human cells, but α-toxin has greater potency and is usually produced in higher concentrations in vitro than PVL.
2. PSMs are small molecular weight cytolysins that include the most active α-type PSMs, but other peptide cytotoxins as well [15]. These toxins are redundantly produced by CA-MRSA strains. As suggested in the Hongo et al. manuscript, when produced PSMs are likely to contribute to PMN toxicity, and thus serious disease, as a function of amplification of the activity of other cytolysins.
3. Importantly and as emphasized by the Hongo et al. study, most of published studies of serious diseases associated with CA-MRSA have been performed in mice, without determination of whether or not studies in mice duplicate human diseases. Mice have been used primarily in many pathogenesis studies due to the availability of inbred

strains and their being inexpensive, but these properties should not be the guiding factors.

Thus far, I have addressed the possible contribution of cytolysins to serious pulmonary diseases, but not the potential contribution of superantigens. There is strong evidence to suggest that mice are highly resistant to the lethal effects of superantigens [18–20]; we have injected Balb/c mice with 3.5 mg of TSST-1 without demonstrable lethal effect, whether administered as bolus injections or in mini-osmotic pumps for continuous daily release. Superantigens, and notably TSST-1, SEB, and SEC, are causes of highly severe human TSS [21–23]. Furthermore, a published study suggest that the lethal dose of superantigens may be as low as 0.1 ug/human [24] (humans are minimally 10^{11} more susceptible to superantigens than Balb/c mice).

In the initial studies of the four pediatric deaths, 2/4 of the CA-MRSA strains produce SEB, and the other 2/4 produce SEC [2]. CA-MRSA USA300 strains produce SE-l Q [25], a novel superantigen that is the prototype of a recently described large group of strains [26], and many a deletion variant of TSST-1 [5]. CA-MRSA USA100 and 200 strains produce TSST-1.

In order to assess the role of superantigens in CA-MRSA serious pulmonary infections, it is critical to evaluate the strains in models other than mice. Studies suggest that rabbits are an important model system [27–29]. In our studies in rabbits with use of the isogenic USA400 strains from the DeLeo, we have shown that SEC is critical both to necrotizing pneumonia and lethality (Strandberg, K.L. and Schlievert, P.M. 2008. Staphylococcal superantigens contribute to necrotizing pneumonia in rabbits. American Society for Microbiology Abstract B-055). We also concluded that PVL is not critical. Our studies of a CA-MRSA USA200 strain (α-toxin$^-$, γ-toxin$^-$, and PVL$^-$, TSST-1$^+$), in rabbits either immune to TSST-1 or not immune to TSST-1, demonstrate that TSST-1 is critical for both necrotizing pneumonia and fatal disease, and that pore-forming cytolysins are not critical.

Our studies have not determine why some patients develop necrotizing pneumonia, others develop purpura fulminans, and still others develop TSS. Differences may result from heterogeneity in humans and differences in production of specific virulence factors of CA-MRSA.

In sum, there remains controversy concerning the secreted virulence factors of CA-MRSA causing serious septicemia and pulmonary diseases based on choice of animal models. However, it is likely that production of the various multiply-redundant cytolysins, combined with production of myriad superantigens, leads to these devastating illnesses.

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