Cytolysins, Superantigens, and Pneumonia Due to Community-Associated Methicillin-Resistant Staphylococcus aureus

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(See the article by Hongo et al, on pages 715–23.)

In a 2007 report of a collaborative study that analyzed data from 2005, the Centers for Disease Control and Prevention (CDC) stated that Staphylococcus aureus is the most important cause of serious and fatal infections in the United States [1]. Methicillin-resistant S. aureus (MRSA) strains are well recognized as posing substantial infection problems for hospitals, but in the last 15 years, community-associated MRSA (CA-MRSA) strains have emerged to become highly important causes of skin and soft-tissue infections, as well as septicemia and pulmonary diseases that are often fatal [2–4]. In 1999, there were 4 children reported who had pulmonary infection due to community-associated MRSA—identified as CDC USA400 by use of pulsed-field gel electrophoresis (PFGE)—and all 4 children died [2]. The MRSA isolates recovered from these patients had multiple secreted virulence factors, including cytolyisns (α-toxin, γ-toxin, Panton-Valentine leukocidin (PVL), phenol-soluble modulins [PSMs]) and superantigens (staphylococcal enterotoxin [SE] B or C). In 2003, CA-MRSA CDC USA300 isolates emerged, which were also associated with skin and soft-tissue infections, as well as life-threatening septicemia and pulmonary diseases [3]. Their secreted virulence factors included cytolyisns (α-toxin, γ-toxin, PVL, and PSMs) and a 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 USA200 isolates are also emerging. These isolates have multiple secreted virulence factors, including combinations of cytolyisns (α-toxin, γ-toxin, PVL, and PSMs) and superantigen TSST-1 (a significant emergent strain in this category has the following phenotype: for cytolyisns, α-toxin, γ-toxin, PVL, and PSMs; as well as superantigen TSST-1). It is important to remember that methicillin-susceptible versions of these organisms with the same PFGE type exist and that these methicillin-susceptible strains produce the same types of infection.

With the emergence of severe pulmonary diseases caused by CA-MRSA, investigators have assessed the roles of secreted virulence factors. These diseases most often occur after viral infection and in association with preexisting conditions such as asthma, and they represent highly important fractions of CA-MRSA infections. The most widely recognized diseases associated with lung infections include necrotizing pneumonia [2, 3], purpura fulminans [6, 7], and postviral toxic shock syndrome [8].

Cytolysins have been the most studied virulence factors in serious pulmonary infections, including those studied in the article by Hongo et al [9] in this issue of Journal. Much of the research has focused initially on CDC USA300 and USA400 strains. When initially described, these isolates, which were associated with necrotizing pneumonia, were reported to secrete PVL [10, 11]. Numerous clinical studies quickly emphasized the strong association between PVL and illness. There followed multiple studies that aimed to determine whether PVL was a significant virulence factor that played a role in the causation of necrotizing pneumonia or is simply a
“tag-along” marker for CA-MRSA strains. The first of these studies, reported by the research group of Voyich et al., analyzed disease production in mice by use of isogenic CA-MRSA strains that differed only in their PVL production [12]. Their study demonstrated that PVL did not play a critical role in disease production, because both PVL+ and PVL− strains caused comparable illnesses. The authors also showed that PVL+ and PVL− strains lysed human PMNs at a comparable rate. Their paper was followed by a collaborative study by Labandeira et al. [13], which showed that in mice, PVL was critical to disease production, thus creating a significant controversy. More recently, Voyich et al. teamed up with Bubeck Wardenburg et al. and examined the role of other cytolysins in serious pulmonary diseases [14–16]. These studies in mice convincingly demonstrated that both α-toxin and PSMs, but not PVL, are key participants in serious CA-MRSA illnesses. Finally, the study by Hongo et al. [9] has evaluated the potential roles of both PVL and PSMs in serious diseases by studies of cytolysin effects on mouse and human peripheral blood mononuclear cells (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.

Thus, 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 the results from the murine studies mentioned above are discrepant. It is unclear how PVL can lack importance in serious disease in mice in one study [12], and yet be found to be critical to disease in a later study [13]. The study by Hongo et al. [9] does not resolve this controversy, but it appears to support the findings of Labandeira et al. [13]. PVL is a hetero-chain, heptamer pore-forming cytotoxin that belongs to the larger γ-toxin cytolysin family [17]. Similarly, α-toxin is well established as a potent human cell cytotoxin; this toxin is a homo-chain, heptamer pore-forming toxin [18]. All of these cytolysins (PVL, α-toxin, and γ-toxin [not evaluated in any of the above studies]) have cytotoxic activity against humans cells (including PMNs and epithelial cells) and form pores of the same approximate size, but differ somewhat in their cytotoxic potency depending on the 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 the amount produced and its potency. In our laboratory, 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.

PSMs are small molecular weight cytolysins that include the most active α-type PSMs, as well as other peptide cytolysins [16]. These toxins are redundantly produced by CA-MRSA strains. As suggested in the article by Hongo et al. [9], PSMs are likely to contribute to PMN toxicity and thus to serious disease by amplifying the activity of other cytolysins.

Importantly, and as emphasized by the Hongo et al. study [9], most published studies of serious disease associated with CA-MRSA have been performed in mice without determining whether studies in mice duplicate human disease. Mice have been used in many studies of pathogenesis primarily because of the availability of inbred strains and the fact that mice are inexpensive, but these properties should not be the guiding factors in choosing a study model.

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 [19–21]. We have injected BALB/c mice with 3.5 mg of TSST-1 without demonstrable lethal effect, whether administered as bolus injections or by use of miniosmotic pumps for continuous daily release. Superantigens, and notably TSST-1, SEB, and SEC, cause very severe TSS in humans [22–24]. Furthermore, a published study suggested that the lethal dose of superantigens in humans may be as low as 0.1 µg [25] (humans are minimally 104 times more susceptible to superantigens than BALB/c mice).

In the initial studies of the 4 pediatric deaths, 2 of 4 of the CA-MRSA strains produced SEB, and the other 2 produced SEC [2]. CA-MRSA USA300 strains produce SE-I Q [26], a novel superantigen that is the prototype superantigen made by a recently described large group of strains [27], and many have a deletion variant of TSST-1 [5]. CA-MRSA USA100 and USA200 strains produce TSST-1.

To assess the role of superantigens in serious pulmonary infections due to CA-MRSA, it is critical to evaluate the strains in models other than mouse models. Rabbits may offer an important model system [28–30]. In our studies in rabbits that used the isogenic USA400 strains from the DeLeo group, we have shown that SEC is critical both for necrotizing pneumonia and lethality [31]. We also concluded that PVL is not critical in this model. Our studies of a CA-MRSA USA200 strain (phenotype α-toxin−, γ-toxin−, PVL−, and TSST-1−) in rabbits either immune to TSST-1 or not immune to TSST-1, demonstrated that TSST-1 is critical for both necrotizing pneumonia and fatal disease, and that pore-forming cytolysins are not critical.

Studies have not determined why some patients develop necrotizing pneumonia, others develop purpura fulminans, and still others develop TSS. These differences may result from heterogeneity in humans and differences in production of specific virulence factors of CA-MRSA.
In sum, researchers’ choices regarding animal models have led to controversy about whether the secreted virulence factors of CA-MRSA cause serious septicemia and pulmonary diseases. However, it is likely that production of various multiply redundant cytolsins combined with the production of myriad superantigens leads to the devastating illnesses observed.

References


