Correspondence

Response to Voyich et al.

To the Editor—The article by Voyich et al. [1] highlights the need to understand the pathogenesis of infection by community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). However, their investigation was inadequate to support the conclusion that Panton-Valentine leukocidin (PVL) is not the major virulence determinant of CA-MRSA.

The in vivo end points used in this investigation—abscess size and early lethality after intravenous injection of S. aureus—are primarily determined in mice by α-toxin [2]. The elimination of α-toxin from S. aureus reduces the size of subcutaneous abscesses in mice at least 10-fold, compared with infection with the parent strain [2]. Whereas the LD₅₀ of α-toxin in mice is only 1 μg [3], PVL is nonlethal even at high doses [4]. In humans, anti-staphylococcal antibodies are found in cord blood and in virtually everyone >18 weeks old [5]. α-toxin injected directly into human forearms elicits a biphasic cutaneous response attributed to interactions between antigen and antibody rather than toxicity [6], and passive immunization with human serum protects mice against death during parenteral S. aureus infection [7, 8]. Finally, α-toxin exerts multiple effects on human polymorphonuclear cells, including cell damage [9].

In summary, staphylococcal infection in humans typically arises despite opsonizing and α-toxin–neutralizing humoral immunity, which was not present to reduce the dominant effects of α-toxin in this investigation. It is premature to acquit PVL, a toxin produced by <5% of S. aureus before the emergence of CA-MRSA [10], as the major virulence determinant that now promotes CA-MRSA disease by affecting the delicate balance between S. aureus and humans without much preexisting immunity to PVL.

How Relevant Were the Models Used to Measure the Impact of Panton-Valentine Leukocidin in Human Staphylococcal Infections?

To the Editor—Voyich et al. [1] reported findings that led them to assert “[PVL] is not the major determinant of disease caused by these prominent CA-MRSA strains” (p. 1769). The 3 lines of evidence supporting this assertion contain enough methodological uncertainties to make such a conclusive statement premature at best.

First are the limitations inherent in the murine sepsis model, especially when applied to the issue at hand. Mouse leukocytes are not lysed by Panton-Valentine leukocidin (PVL) as they are in rabbits and humans. Also, in the mouse sepsis model, mice are relatively resistant to intravenous bacterial challenges, which necessitates the use of high concentrations over relatively short periods of time [2, 3]. The immediate response to bacterial cell-wall constituents in this model is likely to obscure the contributions of secreted toxins.

Second, in the murine and rabbit abscess models, according to Voyich et al., direct inoculations of up to 1 × 10⁷ organisms produced abscesses in 4 days that resolved on their own in 2 weeks. The difference between this picture and the rapid evolution (“spider bites” or necrotizing fasciitis) seen in human infections, which are caused by much smaller inocula, raises as many questions about the animal models as they do about the contribution of PVL. Toxin production or secretion may be down-regulated under conditions of high bacterial density or other environmental factors [4].

Third, the results of in vitro leukocyte lysis experiments, which used isogenic Δpvl...
strains of USA300 and USA400 and conditioned cell-culture medium, raise more questions than they answer, particularly given that they seem to contradict previous observations in human leukocytes, demonstrating pore formation, down-regulation of oxidative burst, and binding to mitochondrial membranes by the intact toxin in vitro [5]. Failure to demonstrate differences in pore formation or cell lysis after phagocytosis in cell culture is an interesting observation that may be related to PVL regulation during these host-cell interactions. After all, the authors’ post- phagocytosis polymerase chain reaction data indicate a much lower up-regulation of PVL transcripts, compared with that of other lytic enzymes measured under their cell-culture conditions—a fact that is not mitigated by the demonstration that immunoreactive toxin molecules are produced under more favorable culture conditions. Far from diminishing the relevance of PVL in these infections, their conditions. Indeed, their interest in our recent publication [3]. Indeed, α-toxin (α-hemolysin or Hla) may be a significant component of the pathogenesis of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA), but this speculative notion remains to be tested. A prominent role of α-toxin would support our conclusion that Panton-Valentine leukocidin (PVL) is not the major determinant of CA-MRSA virulence. In our study, there were noted differences in the pathologic features of subcutaneous infections among the 9 CA-MRSA strains tested (dermonecrotic lesions vs. abscesses) that might be attributed to varied levels of α-toxin production. Consistent with this idea, the USA300-0114 strain used in our study (also called “Los Angeles County clone” [LAC]) produces significantly more α-toxin in vitro (nearly 10-fold more) than the MW2 strain [4] and causes far more dermonecrosis within 24–48 h after inoculation [5]. By comparison, MW2 causes more rapid death in a sepsis model, and subcutaneous infection almost always causes a bona fide abscess rather than a necrotic lesion [3]. Although one could speculate that a single virulence molecule is solely responsible for any given phenotype or disease, these data, along with those from many other groups, clearly indicate that multiple factors contribute to the pathogenesis of CA-MRSA.

On the other hand, we disagree with Kernodle’s argument that our study was inadequate. Key details of our study and many published studies are at variance with his hypothesis and were overlooked. First, the role played by α-toxin in CA-MRSA virulence remains to be determined, given that the strains used by Patel et al. [5] were neither CA-MRSA nor related to such phylogenetic lineages. Notably, the in vivo end points of the S. aureus infection models used in our study were different from those used by Patel et al. This is because the work by Patel et al. used mouse intraperitoneal inoculation rather than intravenous infection, and they measured skin lesions 24 h after subcutaneous inoculation rather than abscesses, which typically take several days to develop fully in mice [3, 6]. The lesions described by Patel et al. are characteristic of those preceding dermonecrosis [3, 6], a phenomenon known to be associated with α-toxin (and observed with strain LAC but not MW2). In addition, that study used an inoculum of S. aureus at least 5 times that used in our study to achieve a similar lethal effect [3, 5].

Second, Kernodle’s idea relating to α-toxin—neutralizing humoral immunity and lack of preexisting immunity to PVL is speculation and is at variance with published studies [7]. Studies by Lack and Towers [7] demonstrated that only 48% of individuals with confirmed S. aureus infections have high titers of anti–α-toxin antibody, compared with the 70% who have high titers of anti-PVL antibody. According to Lack and Towers, “anti-leukocidin levels rose more consistently than anti–α-haemolysin” (p. 1229) [7]. Inasmuch as the LukS components of γ-hemolysin and PVL are 77% identical [8] (see comparisons of the published sequences in MW2), the high levels of anti-PVL antibody might be due, in part, to cross-reactivity of the anti-Hlg antibody, which is known to occur at relatively high titers in humans [9]. In any case, a significant population of individuals lack

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**References**


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**Reply to Kernodle and to Schwartzman et al.**

To the Editor—We thank Dr. Kernodle [1] and Dr. Schwartzman et al. [2] for their interest in our recent publication [3]. Indeed, α-toxin (α-hemolysin or Hla) may be a significant component of the pathogenesis of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA), but this speculative notion remains to be tested. A prominent role of α-toxin would support our conclusion that Panton-Valentine leukocidin (PVL) is not the major determinant of CA-MRSA virulence. In our study, there were noted differences in the pathologic features of subcutaneous infections among the 9 CA-MRSA strains tested (dermonecrotic lesions vs. abscesses) that might be attributed to varied levels of α-toxin production. Consistent with this idea, the USA300-0114 strain used in our study (also called “Los Angeles County clone” [LAC]) produces significantly more α-toxin in vitro (nearly 10-fold more) than the MW2 strain [4] and causes far more dermonecrosis within 24–48 h after inoculation [5]. By comparison, MW2 causes more rapid death in a sepsis model, and subcutaneous infection almost always causes a bona fide abscess rather than a necrotic lesion [3]. Although one could speculate that a single virulence molecule is solely responsible for any given phenotype or disease, these data, along with those from many other groups, clearly indicate that multiple factors contribute to the pathogenesis of CA-MRSA.