Correspondence

**Does β-toxin Production Contribute to the Cytotoxicity of Hypervirulent Staphylococcus aureus?**

TO THE EDITOR—The recent article by Salgado-Pabón et al, which reported that excision of the staphylococcal β-toxin–converting bacteriophage ϕSa3 is common in ϕSa3-positive strains, was interesting [1]. By demonstrating that β-toxin production is selected for by in vivo infection and enhances virulence in rabbit models of pneumonia and infective endocarditis, Salgado-Pabón et al identified a previously unsuspected role for β-toxin in ϕSa3-positive *Staphylococcus aureus*. Regarding infective endocarditis, they insightfully predicted that the increase in vegetation size associated with β-toxin production could be linked to its nucleo-protein ligase activity. However, other mechanisms by which β-toxin could contribute to pathogenesis in these models were not discussed. Interestingly, β-toxin has been shown to participate in the intracellular virulence of *S. aureus* [2]. β-toxin production, along with production of phenol-soluble modulins, is reportedly required for the disruption of phagosomal membranes by *S. aureus* after internalization and for the bacterium to gain access to the cytoplasm of the infected cell, eventually triggering cell death. Interestingly, hypervirulent strains such as *S. aureus* USA300 escape phagosomes and are highly cytotoxic despite harboring ϕSa3 [3]. Facing this apparent contradiction, some authors hypothesized that intraphagosomal oxidative stress induces ϕSa3 excision and restores β-toxin production [4]. The finding that ϕSa3 excision is common during in vivo infection adds credulity to this hypothesis, which we have investigated recently.

We used the hemolysis profile analysis on sheep blood agar plates described by Salgado-Pabón et al to quantify the frequency of β-toxin variants in *S. aureus* populations. Functional β-toxin production was confirmed using a CAMP test.

![Figure 1](https://academic.oup.com/jid/article-abstract/211/5/846/2912127)
and polymerase chain reaction analysis was performed to assess phage excision and the restoration of the native β-toxin gene, as described elsewhere [5]. We first determined whether ϕSa3 excision was induced under oxidative stress conditions in vitro. Incubation of the hypervirulent S. aureus strain USA300-SF8300 for 3 hours in brain-heart infusion (BHI) broth containing H$_2$O$_2$ at the phage-inducing concentration of 1 mM [6] moderately increased the frequency of β-toxin–producing variants, compared with incubation in BHI broth alone (Figure 1A). As indicated by Salgado-Pabón et al, detection of β-toxin–producing variants can be difficult in the presence of α-toxin. We thus reproduced the experiments with an α-toxin–negative (Δhla) mutant of strain SF8300 (kindly provided by B. A. Diep) and observed the same pattern (Figure 1A). Of note, the basal rate of ϕSa3 excision in strains SF8300 and SF8300Δhla was approximately 1%, which is consistent with the rates reported in strain MW2 [1].

We then investigated whether ϕSa3 excision was induced after the intracellular infection of eukaryotic cells by S. aureus. We used a well-established in vitro model of intracellular infection of MG-63 human osteoblastic cells, with a multiplicity of infection of 100 [3]. In this model, the proportion of β-toxin–producing variants in SF8300 and SF8300Δhla recovered after 6 hours of intracellular infection (ie, ≥3 hours of intracellular localization, after subtracting 2 hours of coculture and 1 hour of gentamicin-based selection of intracellular bacteria [3]) did not differ significantly from that found in bacteria incubated for the same amount of time in cell culture medium alone. These findings suggest that intracellular infection does not induce ϕSa3 excision.

Finally, we determined whether the ability of strains SF8300 and SF8300Δhla to induce infected MG-63 cell death was influenced by β-toxin production. Spontaneous β-toxin–producing variants of SF8300 and SF8300Δhla (3 randomly selected variants per strain) were used along with the parental strains to infect MG-63 cells. Intracellular infection was prolonged for 24 hours, after which cytotoxicity was assessed using a lactate dehydrogenase–based assay. The cytotoxicity of the β-toxin–producing variants did not differ from that of the wild-type strain in both SF8300 and SF8300Δhla (Figure 1B), which is evidence against the contribution of β-toxin to intracellular virulence in this model.

Overall, these data do not support either the induction or involvement of β-toxin during the intracellular infection of MG-63 cells by S. aureus USA300-SF8300 [3]. Hence, the contribution of β-toxin to ϕSa3-positive S. aureus pathogenesis in vivo, which was firmly established by Salgado-Pabón et al, is not likely related to host cell invasion. The consequences of this observation are 2-fold. First, our findings suggest that β-toxin–related pathogenesis occurs in the extracellular compartment, which is in agreement with the mechanisms proposed by Salgado-Pabón et al [1]. Second, these results indicate that the cytotoxicity of hypervirulent S. aureus does not require β-toxin production. Further studies are warranted to determine how β-toxin contributes to S. aureus pathogenesis in vivo and to decipher the mechanisms by which hypervirulent S. aureus kills infected cells in a β-toxin–independent fashion.

Notes

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References

1. Salgado-Pabón W, Herrera A, Vu BG, et al. *Staphylococcus aureus* β-toxin production is common in strains with the β-toxin gene inactivated by bacteriophage. J Infect Dis 2014; 210:784–92.

2. Giese B, Głowinski F, Paprotka K, et al. Expression of delta-toxin by *Staphylococcus aureus* mediates escape from phagosomes of human epithelial and endothelial cells in the presence of beta-toxin. Cell Microbiol 2011; 13:316–29.

3. Rasigade JP, Trouillet-Assant S, Ferry T, et al. PSMs of hypervirulent *Staphylococcus aureus* act as intracellular toxins that kill infected osteoblasts. PLoS One 2013; 8:e63176.

4. Fraunholz M, Sinha B. Intracellular *Staphylococcus aureus* live-in and let die. Front Cell Infect Microbiol 2012; 2:43.

5. Kumagai R, Nakatani K, Ikeya N, Kito Y, Kaidoh T, Takeuchi S. Quadruple or quintuple conversion of *hlb*, *sak*, *sea* (or *sep*), *scn*, and *chp* genes by bacteriophages in non-beta-hemolysin-producing bovine isolates of *Staphylococcus aureus*. Vet Microbiol 2007; 122: 190–5.

6. Selva L, Viana D, Regev-Yochay G, et al. Killing niche competitors by remote-control bacteriophage induction. Proc Natl Acad Sci U S A 2009; 106:1234–8.

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