**Staphylococcus aureus** Leukotoxin GH Promotes Inflammation

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**Background.** *Staphylococcus aureus* produces numerous molecules that facilitate survival in the host. We recently identified a novel *S. aureus* leukotoxin (leukotoxin GH [LukGH]) using proteomics, but its role in virulence remains unclear. Here we investigated the role of LukGH in vivo.

**Methods.** We tested cytotoxicity of LukGH toward polymorphonuclear leukocytes (PMNs) from mice, rabbits, monkeys, and humans. LukGH was administered to mice, rabbits, and a cynomolgus monkey by subcutaneous or intradermal injection to assess cytotoxicity or host response in vivo. The effects of LukGH in vivo were compared with those of Panton-Valentine leukocidin (PVL), a well-characterized *S. aureus* leukotoxin. The contribution of LukGH to *S. aureus* infection was tested using mouse and rabbit infection models.

**Results.** Susceptibility of PMNs to LukGH was similar between humans and cynomolgus monkeys, and was greater than that of rabbits, which in turn was greater than that of mice. LukGH or PVL caused skin inflammation in rabbits and a monkey, but deletion of neither *lukGH* nor *lukGH* and *lukS/F-PV* reduced severity of USA300 infections in rabbits or mice. Rather, some disease parameters (eg, rabbit abscess size) were increased following infection with a *lukGH* and *lukS/F-PV* deletion strain.

**Conclusions.** Our findings indicate that *S. aureus* leukotoxins enhance the host inflammatory response and influence the outcome of infection.

*Staphylococcus aureus* is a prominent cause of bacterial infections worldwide. The pathogen produces a variety of molecules that presumably facilitate survival in or on the human host (reviewed in [1]). Bicomponent, pore-forming leukotoxins are among the secreted molecules produced by *S. aureus*. These molecules have cytolytic activity toward leukocytes, most notably polymorphonuclear leukocytes (PMNs) and mononuclear phagocytes. Some of these leukotoxins, such as Panton-Valentine leukocidin (PVL), are encoded on mobile genetic elements such as prophage, whereas others are encoded by genes located in the core genome and are thus present in the majority of strains [2–4]. Bicomponent leukotoxins are composed of S and F subunits that oligomerize and assemble into a β-barrel pore in the plasma membrane of host cells [2, 5].

Recently we and others described a new member of the leukotoxin family—LukGH (named LukAB by DuMont et al [6])—that is an abundant surface-associated and freely secreted protein of *S. aureus* [3]. Although these studies demonstrated that LukGH causes lysis of phagocytes in vitro and promotes *S. aureus* colonization and survival in the mouse, our understanding of the role of this molecule during *S. aureus* infection remains incomplete [3, 6]. To address this deficiency in knowledge, we tested the ability of purified LukGH to elicit an inflammatory response in the skin of mammals (monkey, rabbit, and mouse) and evaluated contribution of the toxin to the severity of *S. aureus* infection in mice and rabbits.

**MATERIALS AND METHODS**

**Ethics Statement**

All animal studies were approved by the animal care and use committee at Rocky Mountain Laboratories,
National Institute of Allergy and Infectious Diseases (NIAID), and conformed to guidelines of the National Institutes of Health (NIH). Human heparinized blood was obtained from healthy individuals in accordance with a protocol approved by the institutional review board for human subjects of NIAID. Informed consent was obtained from each person who participated in the study.

**Bacterial Strains and Culture Conditions**

USA300 wild-type and isogenic gene deletion strains, LAC, LACΔlukGH, LACΔlukGHΔpvl, LACΔpvl, LACΔhlgABC, and SF8300ΔhlgABC/ΔlukDEΔpvl (provided by Binh An Diep, University of California, San Francisco), have been described elsewhere [3, 7–11]. Unless stated otherwise, bacteria were cultured in trypticase soy broth (Difco) at 37°C with shaking at 225 rpm. Overnight cultures were used directly or diluted 1:200 into fresh medium, and bacteria were cultured to the desired phase of growth—early stationary phase for the abscess model and mid-logarithmic phase for the mouse bacteremia model.

**Purification of LukGH**

LukGH was purified from the culture supernatant of *S. aureus* strain SF8300ΔhlgABC/ΔlukDEΔpvl containing the plasmid pTX-15-lukGH. The pTX15-lukGH plasmid contains the complete open reading frame encoding LukGH and was electroporated into SF8300ΔhlgABC/ΔlukDEΔpvl for the specific purpose of increasing the yield of LukGH in culture supernatants. Briefly, culture supernatants were clarified by centrifugation for 10 minutes at 4000 g to pellet bacteria. Clarified culture supernatant was sterilized by filtration, and proteins were precipitated with ammonium sulfate (saturation, 80%) for ≥6 hours at 4°C using constant stirring. Precipitates were collected by centrifugation (15 000 g for 20 minutes at 4°C) and rehydrated in 30 mM sodium phosphate buffer (pH, 6.5; Buffer-1). Proteins were dialyzed overnight against Buffer-1 prior to separation on a HiPrep 16/10 CM FF Sepharose column (GE Healthcare Life Sciences). A linear gradient of 0–1 M of NaCl in Buffer-1 was used to elute proteins, which were then subjected to a Mono S 5/50 GL column (GE Healthcare Life Sciences) using the same buffer system. Purified LukGH was concentrated and stored at −80°C in 0.2 M of NaCl in Buffer-1. Purity of samples was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry (LC-MS/MS) at the Mass Spectrometry and Proteomics Facility (Research Technologies Branch, NIAID, NIH, Rockville, MD). Protein purity was estimated as ≥93% based on number of peptide hits related to the protein of interest. Accordingly, LukH and LukG had the highest Mascot scores (1789 and 953, respectively, vs ≥276 for other potentially present peptides). The concentration of LukGH was determined by BCA Protein assay (Pierce Protein Research Products/Thermo Fisher Scientific).

PVL was purified from the culture supernatant of LACΔhlgABC as described elsewhere [9].

**Purification of PMNs**

Human PMNs were purified from heparinized blood of healthy donors as described elsewhere [10]. PMNs from mice (6–10 weeks old, immunocompetent, strain RML5; RML/NIAID/NIH, Hamilton, MT) and cynomolgus macaques were purified from heparinized blood using anti-Ly-6G and CD66abc MicroBead kits (MACS Cell Separation Technology, Miltenyi Biotec) according to the manufacturer’s protocols. To obtain sufficient mouse PMNs for each experiment, blood was pooled from 5 mice. PMNs were isolated from venous blood of New Zealand White (NZW) rabbits and humans using standard methods [10–12]. Alternatively, human PMNs were purified using the CD66abc MicroBead kit (MACS Cell Separation Technology, Miltenyi Biotec). Methods for isolation of rabbit and mouse PMNs yielded approximately 75% PMNs as determined by flow cytometry. Purity and viability of human and monkey PMNs were ≥97%.

**PMN Assays**

PMN lysis was determined by release of lactate dehydrogenase (LDH) using the Cytotoxicity Detection kit (Roche Applied Sciences) according to the manufacturer’s protocol. Assays were performed with approximately 3.3 × 10⁵ PMNs per well in a 96-well culture plate as described elsewhere [11, 12]. Permeabilization of the PMN plasma membrane (pore formation) by LukGH or PVL was assessed by uptake of ethidium bromide as described elsewhere [3, 9]. A gate on PMNs was used for analysis of flow cytometry data. Preliminary data indicated that purification using the microbead method (described above) did not affect pore formation or LDH release.

**Animal Infection Models**

Mouse and rabbit *S. aureus* skin infection models and the mouse bacteremia model have been described elsewhere [7, 13]. For the mouse bacteremia model, animals were monitored initially every 3 hours for the first 72 hours and then every 8 hours thereafter for up to 120 hours. Animals were euthanized if they were unable to eat or drink or if they became immobile. Criteria for determining morbidity and sickness in mice included hunched posture, decreased activity, ruffled fur, and labored breathing. Near mortality was scored as lethal in this model and these mice were euthanized, since in our experience mice that meet the criteria for euthanasia almost always die within a few hours.

**Skin Toxicity**

The host response to purified LukGH or PVL was tested in mice (strain Crl:SKH1-E; Charles River), rabbits (NZW, strain Cr1c:KBL), and a cynomolgus macaque (born in-house in
October 1996; Rocky Mountain Laboratories, Hamilton, MT). Two rabbits per protein per type of injection (4 total) were shaved and 0, 3, 30, 100, 300, 1000, or 3000 ng of purified LukGH or PVL was administered by subcutaneous or intradermal injection in 0.05 mL of sterile Dulbecco phosphate-buffered saline (DPBS). Thus, each rabbit received 7 injections, including one containing DPBS. Mice received LukGH or PVL by subcutaneous injection of a single bolus of each amount (3–3000 ng) into the right flank. Three mice per dose per protein were used (36 animals total). One male cynomologus macaque was used to test the host response to 0, 3, 100, and 1000 ng of LukGH or PVL in 0.05 mL of sterile DPBS. Animals were evaluated at 3 hours after injection and then monitored daily for up to 14 days. The area of inflammation was measured daily with a caliper. Length (L) and width (W) values were used to calculate area (\(A = \pi \cdot L/2 \times W/2\)).

**Histopathology Analysis and Recovery of *S. aureus* From Abscesses**

Histopathology was performed on lesions excised on day 1 and day 3 following intradermal injection of 1000 ng of LukGH or PVL. Tissue samples were prepared and stained with hematoxylin-eosin as described elsewhere [13, 14], and tissues were evaluated by a veterinary pathologist (D. J. G.). Images were adjusted for brightness and contrast using Adobe Photoshop CS5 (Adobe Systems).

An additional set of 8 animals (2 rabbits per strain) was used to evaluate whether deletion of *lukGH* and/or *lukS/F-PV* in USA300 altered the number of colony-forming units (CFUs) recovered from abscesses. We chose day 6 after infection on which to compare recovered CFUs, because abscesses are well-formed at this time and are often at or near peak size [13]. Animals were infected as described above, and on day 6 they were euthanized and abscesses with surrounding tissue were surgically removed. Abscesses were weighed and homogenized in 10 mL of DPBS using OmniTHQ digital tissue homogenizer (OMNI International). Ten-fold serial dilutions were plated on trypticase soy agar (TSA) plates and bacterial CFUs were enumerated after 24 hours of incubation at 37°C. A DPBS injection site was used as a control, and no growth was recorded on TSA plated after the 24-hour incubation period.

**RESULTS AND DISCUSSION**

**Cytotoxicity of LukGH Toward Human PMNs**

Our previous studies and those of DuMont et al used culture supernatants from *S. aureus* wild type and Δ*lukGH* isogenic mutant strains or recombinant protein to demonstrate LukGH is a pore-forming toxin [3, 6]. However, data with culture supernatants provide indirect evidence for toxin activity and recombinant proteins may underestimate biological activity. Therefore, to gain further insight into a possible role played by LukGH during *S. aureus*-host interaction, we evaluated the ability of purified LukGH to form pores in PMN plasma membranes and ultimately cause cell lysis (Figure 1). For these assays, we purified LukGH from *S. aureus* culture supernatants, and purity was assessed by SDS-PAGE and mass spectrometry (Figure 1A and data not shown).

LukGH-mediated pore formation was time and concentration dependent (eg, pore formation in human PMNs caused by 1 nM LukGH was 10.3% at 15 minutes and 70.0% at 30 minutes) (Figure 1B and 1C). Inasmuch as the ability of PVL to form pores in the PMN plasma membrane is well characterized, we also compared pore-forming capacity of LukGH with that of purified PVL (Figure 1B and 1C). In general, LukGH and PVL had similar capacity to cause formation of plasma membrane pores in human PMNs by 30 minutes, but there were differences under some assay conditions (Figure 1B and 1C). For example, LukGH-mediated pore formation was significantly greater than that caused by PVL in assays utilizing 0.5 nM leukotoxin (pore formation was 46.3% for LukGH vs 21.5% for PVL; *P < .001*). On the other hand, pore formation caused by 1 nM PVL was more rapid than that caused by 1 nM LukGH (*P < .0001*), albeit there was no significant difference by 30 minutes (Figure 1B). The time- and concentration-dependent ability of LukGH to cause plasma membrane pores in PMNs was largely mirrored by cell lysis (Figure 1D).

**Species Specificity**

It is known that staphylococcal leukotoxins exhibit species or target cell specificity in vitro. Whether these attributes in vitro have any bearing on the role of the toxin during infection in vivo remains unclear, but such information should be considered in the context of animal infection models. To determine whether PMN susceptibility to LukGH differs among selected animal species, we compared the ability of LukGH to cause formation of plasma membrane pores in PMNs from humans, nonhuman primates (cynomolgus macaques or monkeys), rabbits, and mice (Figure 2A). PMNs from peripheral blood of humans and monkeys were similarly highly susceptible to pore formation caused by LukGH (50% or half-maximum concentration [EC50] for human PMNs, 0.25 nM; EC50 for monkey PMNs, 0.5 nM) (Figure 2A). By comparison, PMNs from rabbits (EC50 35 nM) and mice (EC50 550 nM) had reduced susceptibility to LukGH (Figure 2A). Consistent with the pore-formation data, there was concentration-dependent lysis of PMNs from each of the animal species (Figure 2B). We note that the level of PMN lysis failed to increase significantly after 1 hour of incubation, perhaps due to adsorption or binding of the leukotoxin by target cells.

**LukGH-induced Inflammation**

To determine whether LukGH has potential to contribute to skin and soft tissue infections, we tested the ability of purified
LukGH to cause tissue damage and/or an inflammatory response in the skin of mice, rabbits, and a cynomolgus macaque (Figures 3 and 4; Supplementary Figure 1). LukGH (0–3000 ng) was delivered to animals by intradermal (rabbits and monkey) and/or subcutaneous (rabbits and mice) injection. We compared the response to LukGH in these animals with that to purified PVL, which has been shown elsewhere to elicit an inflammatory response in the skin of rabbits but not mice [15–17]. Overt signs of the inflammatory response in these animal models included swelling, redness, and/or localized high skin temperature around injection site, and inflammation—when present—typically peaked within the first 48 hours (Figure 3). No visual systemic effects, including ruffled fur, lack of appetite, or altered mobility, were observed in the animals. Neither LukGH nor PVL induced a noticeable inflammatory response in the skin of mice (Supplementary Figure 1), which is compatible with the reduced susceptibility of mouse PMNs to either of these leukotoxins in vitro (Figure 2 and [15–17]). In contrast, LukGH or PVL caused dose-dependent skin inflammation in rabbits (Figure 3A–D).

LukGH-induced inflammation was readily apparent in animals that received the highest concentrations of leukotoxin by intradermal injection, but it was virtually absent following subcutaneous delivery (compare Figure 3A and 3B with Figure 3C and 3D). Intradermal and subcutaneous injection of PVL resulted in inflammation that was more pronounced compared with that elicited by LukGH (Figure 3A–D). For example, 1000 and 3000 ng of PVL produced areas of inflammation nearly twice those caused by an equivalent amount of LukGH (494 mm² vs 252 mm² for 1000 ng and 857 mm² vs 442 mm² for 3000 ng) (Figure 3B and 3D). The susceptibility of rabbit PMNs to LukGH was reduced compared with that of PVL (approximately 30-fold [15, 18]) (Figure 2A), and correspondingly, purified LukGH had reduced capacity to cause an inflammatory response in rabbit skin (Figure 3A–D).

The inflammatory response to LukGH or PVL in monkey skin was reduced compared with that in rabbits (approximately 2–3-fold less after intradermal administration of 1000 ng of leukotoxin at 24 hours) (Figure 3E). The finding that PVL had relatively limited capacity to cause skin inflammation in the
monkey is consistent with the observations of Löffler et al., who reported that nonhuman primate PMNs have reduced susceptibility to PVL-mediated pore formation compared with rabbit or human PMNs [15]. However, our finding that LukGH had relatively limited capacity to cause skin inflammation in the monkey—albeit the inflammatory response was more protracted than that caused by PVL—is unexpected, since monkey PMNs were highly susceptible to the effects of LukGH in vitro (Figure 2). These data suggest that the susceptibility of PMNs to LukGH is not directly linked to (or correlated with) the ability of the toxin to elicit an inflammatory response. To better understand the inflammatory response elicited by LukGH, we performed histopathology analysis of skin tissue from rabbits that had been administered 1000 ng of LukGH or PVL by intradermal injection (Figure 4). LukGH and PVL each caused inflammation in the dermis, whereas LukGH additionally elicited an inflammatory response in the upper layer of dermis within 24 hours after injection (Figure 4A, 4B, 4D, and 4E). Consistent with the previous report by Cribier et al [16], PVL caused an acute inflammatory lesion in the dermis that contained viable and necrotic neutrophils, and was circumscribed with a dense ring of neutrophils (Figure 4D and 4F). By comparison, inflammatory lesions caused by LukGH were smaller and contained correspondingly fewer neutrophils (Figure 4A–C). On day 3, LukGH-induced lesions were partially resolved, whereas those caused by PVL persisted and had a higher density of cells, mainly neutrophils (Figure 4F and 4L). These data are consistent with the gross pathology results (Figure 3A–D) and the differential sensitivity of rabbit neutrophils to LukGH and PVL in vitro.

Figure 2. Neutrophil sensitivity to leukotoxin GH (LukGH) is species-specific. A, LukGH-mediated pore formation. Polymorphonuclear leukocytes (PMNs) from humans, monkeys, rabbits, or mice were incubated for 30 minutes with 0–2000 nM LukGH. Daggers indicate pore formation on day 2 (eg, mean abscess size [area] for rabbits infected with ΔlukGH/Δpvl was 726 mm² vs 169 mm² for those infected with LAC; P < .05) (Figure 5A). In addition, abscesses caused by the ΔlukGH/Δpvl strain were on average 52% larger than those caused by the wild-type strain on days 3 and 5–11, albeit the differences were not statistically significant (Figure 5A). Despite the trend of increased abscess size in those rabbits, there was similar recovery (ie, no significant difference in CFUs compared with wild-type infected animals) of all S. aureus strains from abscesses on day 6 (Figure 5B). In previous studies, we found that rabbit skin abscesses caused by isogenic USA300 strains lacking genes encoding α-hemolysin, α-type phenol-soluble modulins, or accessory gene regulator were smaller than those caused by the wild-type strain, and correspondingly, fewer CFUs were recovered from those lesions [13]. In contrast, there was no positive correlation between rabbit abscess size and recovered bacteria during the acute stage of disease following infection with ΔlukGH, Δpvl, or ΔlukGH/Δpvl strains (Figure 5A–C). It is possible that there are differences in bacterial burden during the resolution phase of disease (days 10–14), but some of the abscesses had resolved by day 14, and more work is needed to better understand this process. All S. aureus strains tested caused virtually identical abscesses in the mouse skin infection model (Figure 5D). Collectively, these data indicate that neither LukGH nor the combination of LukGH and PVL enhance virulence of USA300 in these skin infection models.

Neither ΔlukGH nor ΔlukGH/Δpvl had decreased capacity to cause lethal bacteremia in a mouse model compared with the LAC wild-type strain (Figure 5E). Rather, survival was...
reduced significantly in mice infected with ΔlukGH/Δpvl ($P = .03$; log-rank test for trend). These findings suggest that LukGH and PVL prime the innate immune system for enhanced clearance of *S. aureus*, a notion compatible with recent data that indicate PVL primes human PMNs for enhanced killing of *S. aureus* [19].

**Concluding Remarks**

*S. aureus* leukotoxins are defined by their cytolytic activity toward leukocytes in vitro, and in this regard LukGH is similar to other 2-component leukotoxins (eg, HlgABC, PVL, or LukDE) [3, 6, 20–22]. These molecules have target-cell specificity, which may also differ among animal species. For example, PVL is known to have greater cytolytic activity toward PMNs from humans and rabbits compared with those from mice in vitro. Here we demonstrated that LukGH also has differential activity toward PMNs from selected mammals in vitro, albeit species specificity was distinct from that of PVL. Although purified LukGH and PVL each elicited an inflammatory response in rabbit and monkey skin, the magnitude of the response did not necessarily correlate with cytolytic activity in vitro (eg, compare PMN data in Figure 2 and [15] with the limited inflammatory response in monkey skin in Figure 3E). Despite the noted cytolytic capacity of LukGH and/or PVL toward rabbit PMNs in vitro, neither toxin enhanced virulence in the rabbit model of USA300 skin infection. In fact, disease caused by USA300 strains containing genes encoding LukGH and/or PVL was less severe compared with isogenic mutant strains lacking one or both toxins. These results have at least 2 important implications. First, the cytolytic capacity of LukGH (or PVL) in vitro has no bearing on the severity of the *S. aureus* infection in animal models. Second and more notably, *S. aureus* leukotoxins have the ability to moderate disease severity, possibly by influencing the host innate immune response, for example, by priming the innate immune system for enhanced clearance of *S. aureus*. These findings suggest that LukGH and PVL prime the innate immune system for enhanced clearance of *S. aureus*, a notion compatible with recent data that indicate PVL primes human PMNs for enhanced killing of *S. aureus* [19].

Figure 3. Leukotoxin GH (LukGH) causes skin inflammation. Inflammatory response in the skin of rabbits following intradermal (i.d.) (A and B) and subcutaneous (s.c.) (C and D) injection of purified LukGH or Panton-Valentine leukocidin (PVL). Images in A and C were taken 24 hours after injection. B and D, quantitation of skin inflammation for 14 days. Results are the mean of 2 rabbits. Note that hair was removed prior to administration of leukotoxins. E, Inflammatory response in the skin of a cynomolgus macaque following intradermal injection of LukGH or PVL as indicated. Results are presented as the area of inflammation from 1 monkey. Abbreviation: PBS, phosphate-buffered saline.
Figure 4. Histopathology of rabbit skin inflammation elicited by purified leukotoxin GH (LukGH) or Panton-Valentine leukocidin (PVL). Representative histopathological sections of the inflammatory lesions caused by intradermal injection of 1000 ng of purified LukGH (A–C and G–I) or PVL (D–F and J–L) are shown. A–F, Day 1; G–L, day 3. In A, D, G, and J, original magnification is ×20. B, E, H, and K show an upper layer of dermis; original magnification is ×1000. C, F, I, and L show cells of the inflammatory lesion; original magnification is ×1000. Black arrowhead, polymorphonuclear leukocyte; red arrowhead, lymphocyte; green arrowhead, mononuclear phagocyte.

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immune system for enhanced function. Such a notion is compatible with data from several recent studies, in which experimental or clinical outcomes are actually worse for infections caused by PVL-negative versus PVL-positive *S. aureus* strains [13, 23–25].

Our understanding of the cellular and molecular basis for leukotoxin-mediated innate immune priming remains incomplete, but PVL has been shown to prime human neutrophils for enhanced production of superoxide, elicit granule exocytosis (which enriches the plasma membrane with numerous receptors involved in the innate immune response), and cause release of proinflammatory mediators such as leukotriene B4 and interleukin 8 [26–28]. More recently, we demonstrated that 1 nM PVL—a sublytic concentration—promotes enhanced binding, uptake, and killing of *S. aureus* by human neutrophils [19]. Based on the findings reported herein, we hypothesize that sublytic concentrations of LukGH also prime neutrophils for enhanced function.

Although the ability of LukGH and/or PVL to moderate disease severity may seem counterproductive for the bacterium, the process could in fact promote long-term commensal

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**Figure 5.** Contribution of leukotoxin GH (LukGH) to virulence in animal models of USA300 infection. A, Rabbit abscesses were measured following subcutaneous inoculation with the indicated strains. Results are the mean of 6–10 abscesses (as indicated) from 3–5 rabbits (2 abscesses per animal). *P* < .05 using a 1-way analysis of variance (ANOVA) and Dunnett posttest. B, A separate set of experiments (2 rabbits per strain and 4 abscesses in total) was used to determine bacterial colony-forming units (CFUs) in abscesses on day 6 after infection. C, Area of rabbit abscesses used for enumeration of CFUs in B. Each symbol represents a data point obtained from a single abscess, and the line indicates the mean. There were no significant differences in CFUs recovered from the mutant strains compared with the wild-type (wt) strain as determined using a 1-way ANOVA and Dunnett posttest. D, Mouse abscess model. E, Mouse bacteremia model. Each animal received 5 × 10^7 CFUs of *Staphylococcus aureus* in 0.1 mL of sterile saline by tail vein inoculation. Results in D and E are the mean of 15 animals per *S. aureus* strain. *P = .03 using a log-rank test for trend.
association of S. aureus with the human host by limiting host mortality. That said, there is probably a fine line between leukotoxin-mediated priming for enhanced host innate immunity and enhanced pathogenesis as a consequence of the leukotoxin-mediated inflammatory response. In any case, more work is needed to better understand how LukGH contributes to the interaction of S. aureus with the human host.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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