Bacterial Survival Amidst an Immune Onslaught: The Contribution of the Staphylococcus aureus Leukotoxins

Francis Alonzo III, Victor J. Torres*

Department of Microbiology, New York University School of Medicine, New York, New York, United States of America

The success of Staphylococcus aureus as a human pathogen is influenced by its ability to elaborate factors that prevent infection resolution by the host immune system. Such immune-altering factors include complement inhibitory molecules, antibody binding proteins, super-antigens, as well as potent cytolytic peptides and pore-forming toxins. Here, we discuss one class of immune cell-targeting toxins, the bi-component leukotoxins. These toxins are believed to form octameric oligomers of alternating subunits on the surface of host cells and insert β-barrel pores into cell membranes leading to osmotic imbalance and cell lysis [1]. We will discuss the reemerging interest in leukotoxins as potent virulence factors with defined cellular effects, as well as challenges that have restricted understanding of their functional activity in vivo, while emphasizing areas of interest for future exploration. In addition, we highlight studies supporting the development of antileukotoxin antibodies and immunization strategies as potential modalities to counter S. aureus infection.

PVL and Beyond: A Reemerging Interest in Immune Cell-Targeting Toxins

The most studied of the leukotoxins produced by S. aureus is the Panton-Valentine Leukocidin (LukSF/PVL). Interest in this toxin stems from its prevalence among current epidemic strains of community-acquired methicillin-resistant S. aureus (CA-MRSA) [2]. Indeed, epidemiological evidence exists to link PVL to a number of diseases including skin and soft tissue infections as well as necrotizing pneumonia, for which CA-MRSA is so notorious [2–6]. Experimentally, assessment of the contribution of PVL to pathogenesis has been plagued by conflicting results, owing to the apparent species specificity of toxin action [7]. Despite these experimental difficulties, PVL has been linked to both necrotizing pneumonia and soft tissue infections using rabbit infection models, although its actual contribution to skin and soft tissue infection remains controversial [8–10]. However, PVL is only one of a family of four additional leukotoxins present in strains causing human disease. These include the gamma hemolysins (HlgAB and HlgCB), leukocidin ED (LukED), and leukocidin AB (LukAB, otherwise known as LukHG [11,12]) (Figure 1). A resurgence of interest in these leukotoxins, which are conserved in a greater percentage of clinical isolates, has led to the discovery of potential distinct roles for each toxin in S. aureus pathogenesis.

Though identified ~10 years ago, LukED had only been evaluated in terms of its in vitro capacity to lyse human and rabbit neutrophils as well as red blood cells [Figure 1] [13,14]. Recently however, LukED has been found to contribute to S. aureus pathogenesis upon murine systemic infection due, in part, to toxin killing of phagocytic leukocytes in vivo [15]. The newly identified leukotoxin LukAB was also shown to contribute to distal tissue colonization upon infection with sublethal doses of MRSA [12]. Additionally, among earlier reports of a role for Hlg in septic arthritis and endophthalmitis [16,17], recent evidence from Malachova and colleagues suggests a role for this toxin in bloodstream infection [18]. Together, these data indicate leukotoxins likely contribute to multiple S. aureus disease states in vivo.

Challenging the Proposition of Strict Functional Redundancy

S. aureus leukotoxins exhibit lytic activity on host neutrophils, although some have greater perceived potency than others (Figure 1). Early studies gave considerable attention to this apparent redundancy in toxin targeting using primary human and rabbit neutrophils [19,20]. However, recent efforts are now moving toward investigation of the full repertoire of cells killed by each leukotoxin, with the premise that each may be unique in both the breadth and specificity of its cellular targets despite significant similarities at the amino acid and structural level. Studies by Holzinger et al. confirmed earlier work describing the lytic capacity of PVL on neutrophils and monocytes, but not lymphocytes [21,22]. In addition, they demonstrated the lytic capacity of PVL on macrophages (Figure 1) [21]. HlgCB is toxic toward neutrophils and macrophages but also exhibits lytic activity on red blood cells [23]. HlgAB, on the other hand, is nontoxic toward human macrophages but is potent on murine macrophages (Figure 1) [23]. LukAB is toxic toward neutrophils, monocytes, macrophages, and dendritic cells, but not the T cell line Jurkat [12]. LukED is active against human and rabbit neutrophils, rabbit red blood cells, as well as murine leukocytes (Figure 1) [13–15]. The subtle differences in leukotoxin activity on specific cell types imply cellular recognition via unique factors. Thus, inferring direct relationships between the potency of one leukotoxin and

Citation: Alonzo F III, Torres VJ (2013) Bacterial Survival Amidst an Immune Onslaught: The Contribution of the Staphylococcus aureus Leukotoxins. PLoS Pathog 9(2): e1003143. doi:10.1371/journal.ppat.1003143

Editor: Virginia Miller, University of North Carolina at Chapel Hill School of Medicine, United States of America

Published February 21, 2013

Copyright: © 2013 Alonzo, Torres. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Research in the Torres Lab is supported by New York University School of Medicine Development Funds, an American Heart Association Scientist Development Grant (09SDG2060036) and National Institutes of Health (NIH) grant R56-AI091856-01A1. FA has been supported by National Institutes of Health (NIH) grants T32-AI007180 and F32-AI098395-01A1. The funders had no role in the decision to publish or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: victor.torres@nyumc.org
another is challenging, as abundance or accessibility of specific cellular targets may vary significantly on host cell surfaces. Deciphering the reasons for varied potencies of the leukotoxins on similar cell types as well as their mechanisms of cellular targeting will prove valuable in future attempts to equate leukotoxin function with pathogenic outcomes.

The Lytic Versus Sublytic Hypothesis

It was recently demonstrated that the killing of host phagocytes during systemic infection of mice with *S. aureus* is dependent on LukED production [15]. Thus, the lytic capacity of LukED is likely a biologically relevant process during *S. aureus* pathogenesis. Other
studies with PVL demonstrate that leukotoxins may also elicit cellular effects at sublytic concentrations (Figure 1) [21,23–20]. Notably, PVL induces inflammasome activation of both mono-
cytes and primary macrophages at sublethal doses [23]. Inflam-
masome activation in this context is believed to contribute to the
inflammatory response and subsequent neutrophil recruitment
during necrotizing pneumonia (Figure 1) [23]. Low concentra-
tions of PVL also appear to prime PMNs for increased bacterial killing by
promoting neutrophil activation (Figure 1) [24]. Unfortunately,
investigation of the influence of sublytic toxin concentrations on
leukocytes in vivo has only been studied using PVL in murine
models [29,30]. Such studies are limited due to an inability of PVL
to lyse murine cells. Thus, while the sublytic effects of PVL on
leukocytes are intriguing, the in vivo consequences of such effects
in the presence of active toxin are not understood. Lending
credence to the hypothesis that S. aureus toxins influence cellular
signaling during infection, the prototypical pore-forming cytotoxin
alpha hemolysin targets macrophores to induce inflammasome
activation in vivo [31,32]. It is possible that within a host both lytic and
sublytic concentrations of the bicomponent leukotoxins are also
countered depending on the site and context of infection.
Studies using active toxins amenable to small animal models (such as LukED) may prove valuable in determining the consequences of such sublytic effects.

Overcoming Species Specificity to Investigate
Leukotoxin Function in Vivo

As mentioned, studies of PVL function in vivo have been
complicated by the species specificity associated with cellular
targeting. PVL has negligible lytic activity on murine neutrophils
but is potent on human and rabbit cells [33]. Similar studies have
demonstrated poor lytic activity of LukAB on murine and rabbit
neutrophils, but potent activity on human neutrophils [12,25].
Interestingly, LukAB still influences the pathogenesis of MRSA in
murine systemic infection models [12]. Future work aimed at
deciphering the mechanism by which LukAB facilitates patho-
genesis in murine models will allow a better assessment of this
protein’s activities in vivo. Additionally, HlgCB kills human
macrophages but exhibits little lytic activity on murine macro-
phages [23]. Thus, studies using murine models to evaluate these
particular toxins in vivo are best interpreted in light of their
nonlytic effects. For this reason, many PVL studies are now
conducted using rabbit models of infection [8,9]. In contrast, LukED is toxic toward murine, rabbit, and human leukocytes and is thus amenable to in vivo studies using murine models of infection [15]. Indeed, the lytic activity of LukED was recapitu-
lated on phagocytic leukocytes in vivo [15]. Thus, future studies of LukED are likely to provide a robust model of leukotoxin function in vivo. The additional finding that HlgAB is lytic on murine macrophages supports assessment of this toxin using murine models [23].

The Leukotoxins as Valuable Vaccine and
Therapeutic Targets

Evidence indicating a role for each of the leukotoxins in the
greater virulence of S. aureus implies potential value in targeting
these molecules to counter infection. Leventie et al. have generated
humanized heavy-chain-only antibodies and diabodies against
PVL that show promise in their ability to neutralize the damaging
effects of the toxin in vivo [34]. These same anti-PVL antibodies also block the activity of HlgCB on host cells [34]. Dual neutralization by these antibodies is perhaps not surprising due to the high degree of sequence similarity among leukotoxins but serves as proof that it is possible to generate single antibodies or a
subset of antibodies with the ability to neutralize multiple
leukotoxins, thereby blunting disease progression [35]. Vaccination of mice with PVL has likewise demonstrated promise toward
reducing the pathogenic outcome of S. aureus infection [36],
though other studies, which passively immunized mice with serum from PVL-immunized rabbits, lead to increased virulence for some strains [30]. In either case, these murine studies should be interpreted with caution given the low level of PVL lytic activity on murine cells. Even so, both the humanized antibody and early-stage
vaccination studies suggest that use of leukotoxins as immunizing agents is a potentially reasonable approach toward promoting natural clearance of infection. Blocking leukotoxin activity will necessitate targeting multiple toxins, as not all strains contain the same leukotoxin profile and each toxin appears to contribute variably to different disease states. A better understanding of leukotoxin mode(s) of action in vivo, inherent redundancies or lack thereof, and the intricacies of cellular
targeting will prove beneficial in our ability to initiate the
development of novel strategies to counter S. aureus infection.

References

1. Menestrina G, Dalla Serra M, Comai M, Coraiola M, Viero G, et al. (2003) Ion
channels and bacterial infection: the case of beta-barrel pore-forming protein
toxins of Staphylococcus aureus. FEBS Lett 552: 54–60.

2. Vandenesch F, Naimi T, Enright MC, Lin A, Nimmo GR, et al. (2003) Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-
Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9: 597–594.

3. Tristan A, Ferry T, Durand G, Dauwalder O, Bes M, et al. (2007) Virulence
determinants in community and hospital-median methicillin-resistant Staphylococcus aureus. J Hosp Infect 65 Suppl 2: 105–109.

4. Gillot Y, Issartel B, Vanhems P, Fournet JC, Lima G, et al. (2002) Association
between Staphylococcus aureus strains carrying gene for Panton-Valentine
leukocidin and highly lethal necrotizing pneumonia in young immunocompetent
patients. Lancet 359: 735–739.

5. Lin A, Piernot Y, Godail-Gamot F, Bes M, Peter MO, et al. (1999) Involvement of Panton-Valentine leukocidin-producing Staphylococcus aureus in
primary skin infections and pneumonia. Clin Infect Dis 29: 1128–1132.

6. Naimi TS, Le-Dell KH, Como-Sabetti K, Borchardt SM, Boreud DJ, et al. (2003) Comparison of community- and health care-associated methicillin-
resistant Staphylococcus aureus infection. JAMA 290: 2976–2984.

7. Vosich JM, Otto M, Mathema B, Braughton KR, Whitney AR, et al. (2006) Is
Panton-Valentine leukocidin the major virulence determinant in community-
associated methicillin-resistant Staphylococcus aureus disease? J Infect Dis 194:
1761–1770.

8. Diep BA, Chan I, Tattevin P, Kajikawa O, Martin TR, et al. (2010) Polymorphonuclear leukocytes mediate Staphylococcus aureus Panton-Valentine leukoci-
din-induced lung inflammation and injury. Proc Natl Acad Sci USA 107: 5587–5592.

9. Lipinska U, Hermans K, Meulemans I, Dumitrescu O, Badizadegan C, et al. (2011) Panton-Valentine leukocidin does play a role in the early stage of Staphylococcus aureus skin infections: a rabbit model. PLoS ONE 6: e22864. doi:10.1371/
journal.pone.0022864

10. Kobayashi SD, Malachowa N, Whitney AR, Braughton KR, Gardner DJ, et al. (2011) Comparative analysis of USA300 virulence determinants in a rabbit
model of skin and soft tissue infection. J Infect Dis 204: 937–941.

11. Ventura CL, Malachowa N, Hammer CH, Nardone GA, Robinson MA, et al. (2010) Identification of a novel Staphylococcus aureus two-component leukotoxin
using cell surface protemics. PLoS ONE 5: e11634. doi:10.1371/journal.
pone.0011634

12. Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, et al. (2011) Characterization of a novel Staphylococcus aureus two-component leukotoxin
producing cell surface proteins. Mol Microbiol 79: 814–825.

13. Morinaga N, Kishi H, Noda M (2003) Purification, cloning and characteriza-
tion of variant LukEF-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. Microbiol Immunol 47: 81–90.

14. Gravel A, Colin DA, Keller D, Girardot R, Monteil H, et al. (1998) Characterization of a novel structural member, LukE-LukD, of the bi-
component staphylococcal leukotoxin family. FEBS Lett 436: 202–208.
15. Alonso III F, Benson MA, Chen J, Novick RP, Shopsin B, et al. (2012) *Staphylococcus aureus* leucocidin ED contributes to systemic infection by targeting neutrophils and promoting bacterial growth in vivo. Mol Microbiol 83: 423–435.

16. Nilsson IM, Hartford O, Foster T, Tarkowski A (1999) Alpha-toxin and gamma-toxin jointly promote *Staphylococcus aureus* virulence in murine septic arthritis. Infect Immun 67: 1045–1049.

17. Supersac G, Piemont Y, Kubina M, Prevost G, Foster TJ (1998) Assessment of the role of gamma-toxin in experimental endophthalmitis using a hlg-deficient mutant of *Staphylococcus aureus*. Microb Pathog 24: 241–251.

18. Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, et al. (2011) Global changes in *Staphylococcus aureus* gene expression in human blood. PLoS ONE 6: e18617. doi:10.1371/journal.pone.0018617

19. Menestrina G, Serra MD, Prevost G (2001) Mode of action of beta-barrel pore-forming toxins of the staphylococcal alpha-hemolysin family. Toxicon 39: 1661–1672.

20. Káneko J, Kamio Y (2004) Bacterial two-component and hetero-heptameric pore-forming cytolysins: structures, pore-forming mechanism, and organization of the genes. Biosci Biotechnol Biochem 68: 981–1003.

21. Holzinger D, Gieldon L, Mysore V, Nippe N, Taxman DJ, et al. (2012) *Staphylococcus aureus* Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. J Leukoc Biol 92(5): 1069–1081.

22. Meunier O, Falkenrodt A, Monteil H, Colin DA (1995) Application of flow cytometry in toxinology: pathophysiology of human polymorphonuclear leukocytes damaged by a pore-forming toxin from *Staphylococcus aureus*. Cytometry 21: 241–247.

23. Perret M, Badiou C, Lina G, Burbaud S, Benito Y, et al. (2012) Cross-talk between *Staphylococcus aureus* leukocidins-intoxicated macrophages and lung epithelial cells triggers chemokine secretion in an inflammasome-dependent manner. Cell Microbiol 14: 1019–1036.

24. Graves SF, Kobayashi SD, Braughton KR, Whitney AR, Sturdevant DE, et al. (2012) Sublytic concentrations of *Staphylococcus aureus* Panton-Valentine leukocidin alter human PMN gene expression and enhance bactericidal capacity. J Leukoc Biol 92: 361–374.

25. Malachowa N, Kobayashi SD, Braughton KR, Whitney AR, Parnell MJ, et al. (2012) *Staphylococcus aureus* leukotoxin GH promotes inflammation. J Infect Dis 206: 1183–1193.

26. Konig B, Prevost G, Piemont Y, Konig W (1995) Effects of *Staphylococcus aureus* leukocidins on inflammatory mediator release from human granulocytes. J Infect Dis 171: 607–613.

27. Colin DA, Monteil H (2003) Control of the oxidative burst of human neutrophils by staphylococcal leukotoxins. Infect Immun 71: 3721–3729.

28. Henler T, Koller M, Prevost G, Piemont Y, Konig W (1994) GTP-binding proteins are involved in the modulated activity of human neutrophils treated with the Panton-Valentine leukocidin from *Staphylococcus aureus*. Infect Immun 62: 5281–5289.

29. Young P, Pier GB (2012) Immune-activating properties of Panton-Valentine leukocidin improve the outcome in a model of methicillin-resistant *Staphylococcus aureus* pneumonia. Infect Immun 80: 2884–2904.

30. Young P, Pier GB (2010) Antibody-mediated enhancement of community-acquired methicillin-resistant *Staphylococcus aureus* infection. Proc Natl Acad Sci U S A 107: 2241–2246.

31. Craven RR, Gao X, Allen IC, Gris D, Bubeck Wardenburg J, et al. (2009) *Staphylococcus aureus* alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytes. PLoS ONE 4: e7446. doi:10.1371/journal.pone.0007446

32. Kebaier C, Chamberland RR, Allen IC, Gao X, Brodie PM, et al. (2012) *Staphylococcus aureus* alpha-hemolysin mediates virulence in a murine model of severe pneumonia through activation of the NLRP3 inflammasome. J Infect Dis 205: 807–817.

33. Lollier B, Hussain M, Grundmeier M, Bruck M, Holzinger D, et al. (2010) *Staphylococcus aureus* panont-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS Pathog 6: e1000715. doi:10.1371/journal.ppat.1000715

34. Laventie BJ, Rademaker HJ, Saleh M, de Boer E, Janssen R, et al. (2011) Heavy chain-only antibodies and tetravalent bispecific antibody neutralizing *Staphylococcus aureus* leukotoxins. Proc Natl Acad Sci U S A 108: 16404–16409.

35. Cheung GY, Otto M (2012) The potential use of toxin antibodies as a strategy for controlling acute *Staphylococcus aureus* infections. Expert Opin Ther Targets 16: 601–612.

36. Brown EL, Dumitrescu O, Thomas D, Badiou C, Koers EM, et al. (2009) The Panton-Valentine leukocidin vaccine protects mice against lung and skin infections caused by *Staphylococcus aureus* USA300. Clin Microbiol Infect 15: 156–164.