Children with Invasive *Staphylococcus aureus* Disease Exhibit a Potently Neutralizing Antibody Response to the Cytotoxin LukAB

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Despite the importance of *Staphylococcus aureus* as a common invasive bacterial pathogen, the humoral response to infection remains inadequately defined, particularly in children. The purpose of this study was to assess the humoral response to extracellular staphylococcal virulence factors, including the bicomponent leukotoxins, which are critical for the cytotoxicity of *S. aureus* toward human neutrophils. Children with culture-proven *S. aureus* infection were prospectively enrolled and stratified by disease type. Fifty-three children were enrolled in the study, of which 90% had invasive disease. Serum samples were obtained during the acute (within 48 h) and convalescent (4 to 6 weeks postinfection) phases, at which point both IgG titers against *S. aureus* exotoxins were determined, and the functionality of the generated antibodies was evaluated. Molecular characterization of clinical isolates was also performed. We observed a marked rise in antibody titer from acute-phase to convalescent-phase sera for LukAB, the most recently described *S. aureus* bicomponent leukotoxin. LukAB production by the isolates was strongly correlated with cytotoxicity *in vitro*, and sera containing anti-LukAB antibodies potently neutralized cytotoxicity. Antibodies to *S. aureus* antigens were detectable in healthy pediatric controls but at much lower titers than in sera from infected subjects. The discovery of a high-titer, neutralizing antibody response to LukAB during invasive infections suggests that this toxin is produced *in vivo* and that it elicits a functional humoral response.

With an estimated incidence of 26 infections per 100,000 persons, *Staphylococcus aureus* is the most common invasive bacterial pathogen in the United States (1), responsible for 2% of all hospital admissions (2). This commensal organism colonizes the nares of approximately one-third of the human population and has the capacity to leave this niche to infect virtually any body site (3). The prevalence of antibiotic-resistant *S. aureus* is increasing in both the community and hospital settings, particularly in the pediatric population (4), and there is an urgent need for improved methods to prevent and treat staphylococcal disease.

An essential component of *S. aureus* infection and virulence is the production of a group of potent cytolytic toxins (5): alpha-hemolysin (alpha-toxin or Hla), phenol-soluble modulins (PSMs), and bicomponent toxins, which include the Panton-Valentine leukokidin (LukSF-PV or PVL), leukokidin ED (LukED), gamma hemolysins (HlgAB and HlgCB), and leukokidin AB (LukAB) (6–8). These toxins are lytic to host immune effector cells, are important for disease pathogenesis, and have been identified as putative vaccine targets (9–11). LukAB (12), also known as LukGH (13), is a recently described bicomponent leukotoxin that promotes *S. aureus* pathogenesis in both *ex vivo* and *in vivo* models of disease (7, 8, 12). While all of the *S. aureus* leukotoxins are secreted, LukAB can also be abundantly found in association with the bacterial cell surface (13), a unique property that appears to be dependent on growth conditions (8). The humoral immune response to this important leukotoxin has not previously been described.

The primary aim of this study was to define the humoral immune response to secreted staphylococcal exotoxins, in particular the bicomponent leukotoxins, following invasive *S. aureus* disease in children. Following the discovery that children with invasive *S. aureus* disease mount a high-titer antibody response to LukAB, we assessed the neutralization capacity of the anti-LukAB antibody response in children with *S. aureus* disease compared to that of healthy pediatric controls.

**MATERIALS AND METHODS**

**Patient enrollment.** This was a prospective cohort study of children (between 6 months and 18 years of age) admitted to the Monroe Carell Jr. Children’s Hospital at Vanderbilt with culture-confirmed *S. aureus* infection identified within the first 5 days of hospitalization. Potential study subjects were identified through daily contact with the Pediatric Infectious Diseases and Hospital Medicine inpatient services from October 2010 to June 2012. Informed consent was obtained, and children were screened for the following exclusion criteria: polymicrobial infection, primary or secondary immune compromise (including long-term oral or parenteral corticosteroids), history of (or current) malignancy, receipt of intravenous immunoglobulin (IVIG) or blood products in the past 12 months, and known history of invasive staphylococcal disease (Fig. 1). Serum samples were obtained immediately upon enrollment in the study (acute-phase sera) and 4 to 6 weeks following enrollment (convalescent-phase sera). Sera were obtained by centrifugation of unheparinized whole blood samples, and sera were stored at −20°C until processing. When available, clinical isolates were also obtained for molecular characteriza-
tion. The study was approved by the Vanderbilt University Medical Center (VUMC) Institutional Review Board.

Healthy control (HC) subjects were recruited from two sources: from an ongoing clinical trial at VUMC in which blood was obtained from healthy siblings of diabetic children for genetic analysis and from same-day surgery at the Monroe Carell Jr. Children’s Hospital at Vanderbilt from subjects undergoing outpatient surgical procedures for noninfectious diagnoses. Healthy controls were selected if they met the following inclusion criteria: age between 6 months and 18 years, no known history of S. aureus disease of any type, no known primary or secondary immune compromise (including long-term oral or parenteral corticosteroids), no history of (or current) malignancy, and no receipt of IVIG or blood products in the past 12 months. Serum samples were obtained from healthy control subjects at the time of enrollment.

Serum antibody measurement by enzyme-linked immunosorbent assay (ELISA). The serum antibody response was measured against 11 specific S. aureus exotoxin antigens: α-hemolysin (Hla), V8 protease, and each subunit of the bicomponent leukotoxins LukAB (LukA and LukB), PVL (LukS-PV and LukF-PV), LukED (LukE and LukD), and the gamma-hemolysins (HlgA, HlgB, and HlgC). Recombinant Hla and V8 protease were purchased in purified form and reconstituted per manufacturer specifications (Sigma). All other toxins were expressed and purified as previously described (8, 14, 15).

Optimal exotoxin concentration for ELISA was determined by crisscross dilution, and indirect ELISA for each antigen was performed as previously described (16, 17). Briefly, purified antigens were diluted in phosphate-buffered saline (PBS) to a concentration of 0.5 μg/ml and bound to 96-well ELISA plates overnight. Wells were then aspirated and blocked at room temperature (RT) for 1 h with 5% nonfat dried milk in Tris-buffered saline. Serial 2-fold dilutions of sera were added to the plate and incubated for 2 h at RT. After plates were washed, horseradish peroxidase (HRP)-conjugated murine monoclonal antibodies against human total IgG diluted 1:1,000 were added, and plates were incubated at RT for 2 h. Next, substrate solution (3,3′,5,5′-tetramethylbenzidine) was added, and plates were incubated at RT. The reaction was stopped at 30 min with 2 M sulfuric acid, and plates were read spectrophotometrically at 450 nm. ELISA titers were measured as the highest serum dilution at which the absorbance value was at least three times the background absorbance of uncoated control wells. Serum depleted of IgG was used as a negative control, and rabbit IgG (with goat-derived HRP-conjugated anti-rabbit IgG) was used to control for nonspecific binding. Samples were run in duplicate, independently on separate days, and a third run was performed if the first two titer values differed by one dilution. Antibody titer results are presented as geometric mean titers (GMT) for a given population unless otherwise specified.

Molecular characterization. Initial determination of methicillin resistance in S. aureus strains was made by the VUMC clinical laboratory. For methicillin-resistant S. aureus (MRSA) isolates, confirmation of methicillin resistance was performed by plating samples onto paired mannitol salt agar plates, with and without 4 μg/ml of oxacillin (Hardy Diagnostics, Santa Maria, CA). After incubation at 37°C for 48 h and at room temperature for 18 h, plates were inspected for yellow colonies indicative of mannlitol fermentation, a characteristic of S. aureus. After subculturing onto tryptic soy agar with 5% sheep blood (Hardy Diagnostics), rapid latex agglutination testing for clumping factor and protein A was performed on all isolates (Staphaurex Plus; Remel). Following phenotypic confirmation of S. aureus, crude genomic DNA was prepared by incubating the isolates with lysostaphin for 1 h at 37°C and heating the samples at 95°C for 15 min. This template DNA was used to detect the presence of the mecA gene, using previously described oligonucleotide primer sequences (18).

Genomic DNA was purified (Wizard SV purification system; Promega, Madison, WI) and used as the template for PCR detection of genes encoding pvd (19), the accessory gene regulator (agr) locus type (20), and lukAB (12). Assignment of staphylococcal cassette chromosome mec (SCCmec) type by ccr and mec complex typing was performed as previously described (21). Repetitive-element, sequence-based PCR (DiversiLab System; bioMérieux) was used to determine strain type and genetic relatedness between strains (22).

Toxin neutralization assays. Human promyelocytic HL-60 cells were cultured in RPMI 1640 (Cellgro), 10% heat-inactivated fetal bovine serum (FBS), and 100 μg/ml penicillin-100 μg/ml streptomycin (Pen/Strep; Cellgro) and allowed to differentiate to neutrophil-like cells for 3 days per standard techniques (23). S. aureus strain Newman was cultured from a single colony in 3 ml of RPMI and Casamino Acids (RPMI-CAS) and grown at 37°C with shaking at 180 rpm. After 24 h, the strain was subcul-
tured 1:100 in 5 ml of RPMI-CAS in a 15-ml tube and cultured for 5 h at 37°C with shaking at 180 rpm. This growth condition is known to stimulate LukAB production (8, 12). Culture filtrates were obtained by centrifugation at 4,000 rpm at 4°C for 15 min with filter sterilization using 0.2-μm-pore-size syringe filters. Supernatants were tested for baseline toxicity against PMN-HL60s. Dose response of supernatant toxicity was assessed by adding supernatant to the cell culture starting at 20% culture supernatant and diluting 2-fold 8 times in 20 μl RPMI-CAS, with a goal 50% lethal dose (LD₅₀) of ~2.5%. To measure the neutralization capacity of patient sera, 10 μl of each serum sample was added in triplicate to 96-well plates. Toxin supernatant diluted 1:1 with RPMI-CAS was added, 10 μl/well, and wells were mixed by pipetting and incubated for 30 min at RT. Wells containing only RPMI-CAS medium served as an un intoxicated control, while culture supernatant alone, sans patient sera, served as a positive toxicity control. PMN-HL60 cells were added to the plate at 80 μl/well (1 × 10⁶ cells/well) and mixed with the serum/supernatant mixture. This mixture was incubated at 37°C and 5% CO₂ for 1 h, and CellTiter (Promega) was added at 10 μl/well to measure cell viability/metabolism. After 2 h of incubation with CellTiter at 37°C and 5% CO₂, plates were read spectrophotometrically at 490-nm absorbance to measure cell survival. Neutralization titers of patient sera were determined by incubating the above-described mixture with serial dilutions of sera beginning with a 1:100 dilution, and the titer was considered neutralizing if ≥70% of the cells were alive at that dilution.

Isolation of primary human PMNs. Blood samples were obtained from anonymous, consenting, healthy adult donors asuffy coats from the New York Blood Center. This study was reviewed and approved by the New York University Langone Medical Center Institutional Review Board.

PMNs were isolated from peripheral blood mononuclear cells (PBMCs) by Ficoll-Paque PLUS gradient. The pellets were subsequently washed with PBS, and PMNs were separated from erythrocytes with 3% dextran (dextran 500; Pharmacosmos) in 0.9% sodium chloride. Remaining erythrocytes were lysed with ACK lysis buffer (Gibco). PMN purity was at 90 to 95% as determined by flow cytometry.

Cytotoxicity of isolates from children with invasive S. aureus infection. Culture filtrates from S. aureus strains responsible for children’s invasive infection were generated as described above. Freshly purified PMNs were plated in 96-well black clear-bottom tissue culture-treated plates (Corning) at 1 × 10⁵ cells/well in a final volume of 100 μl of phenol red-free RPMI (Gibco) supplemented with 10% heat-inactivated FBS. Cells were intoxicated with 10% and 5% (vol/vol) culture filtrates for 1 h at 37°C and 5% CO₂. Controls for 100% viability were composed of PMNs grown in RPMI-CAS, while controls for 100% toxicity were wells containing only 50 μl of supernatant (1). Of the remaining 54, one patient’s legal guardian declined consent, and the remaining 53 children were enrolled in the study. The mean age of children enrolled in the study was 6.7 years (standard deviation [SD], 4.8 years), and 60% were male.

Results

Characteristics of the patient population. During the study period, 102 children were identified with culture-proven S. aureus disease within the first 5 days of their hospitalization. Of these, 48 were excluded from this study based on the exclusion criteria (Fig. 1). Of the remaining 54, one patient’s legal guardian declined consent, and the remaining 53 children were enrolled in the study. The mean age of children enrolled in the study was 6.7 years (SD, 4.8 years), and 60% were male.

Twenty-six patients were being treated for osteomyelitis or septic arthritis, 19 for bacteremia/septicemia, 4 for pneumonia, and 5 with skin or soft tissue infection. Children with osteomyelitis, septic arthritis, pneumonia, and bacteremia/septicemia were stratified as “invasive disease,” and those with skin or soft tissue infections were classified as “noninvasive disease.” The mean age of healthy control subjects was 7.6 years (SD, 4.7 years), and 52% were male.

Molecular characteristics of S. aureus isolates. Nearly half of the S. aureus isolates (48%) were methicillin resistant. The majority of clinical isolates (53%) were classified as the USA300 pulse type. The second-most-common pulse type was USA500 (23%), followed by USA100 (16%). The majority of clinical isolates (87%) possessed agr type I, and the remainder carried agr type II. All of the isolates were positive for lukAB by PCR, and 59% of the isolates were pvl positive. All of the MRSA isolates possessed SCCmec type IV.
Acute-phase and convalescent-phase antibody response to *S. aureus* disease. For the majority of the extracellular proteins tested, there was a statistically significant rise in titer from acute- to convalescent-phase invasive disease, including for LukA, LukB, alpha toxin (Hla), V8 protease (V8P), the two subunits of PVL (LukF-PV and LukS-PV), and the two subunits of LukED (LukE and LukD) (Fig. 2). The bicomponent subunits LukF-PV, LukE, HlgB, and HlgC exhibited a mild rise from acute- to convalescent-phase titers, but not a statistically significant increase (Table 1). Acute-phase and convalescent-phase titers to all antigens were higher in invasive disease than in noninvasive disease (SSTI), but these differences were not statistically significant (data not shown), possibly due to the lower number of samples from patients with noninvasive disease.

**Comparison with healthy controls.** To contrast the antibody response generated by disease to baseline levels in the healthy population, acute-phase and convalescent-phase sera from children with invasive *S. aureus* disease were compared to those from healthy pediatric controls. A low-level antibody response was detected against all antigens tested in sera from healthy controls (Fig. 2).

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**TABLE 1** Humoral response to *S. aureus* exoproteins in paired acute-phase and convalescent-phase sera following invasive disease

<table>
<thead>
<tr>
<th>Antigen</th>
<th>GMT, acute-phase serum</th>
<th>GMT, convalescent-phase serum</th>
<th>Fold increase, convalescent:acute</th>
<th>95% CI of the ratio</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LukA</td>
<td>314.2</td>
<td>735.1</td>
<td>2.34</td>
<td>1.65–3.33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LukB</td>
<td>108.8</td>
<td>350.7</td>
<td>3.22</td>
<td>1.81–5.72</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hla</td>
<td>165.7</td>
<td>327.0</td>
<td>1.97</td>
<td>1.29–3.01</td>
<td>0.002</td>
</tr>
<tr>
<td>V8 protease</td>
<td>39.3</td>
<td>68.7</td>
<td>1.75</td>
<td>1.33–2.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LukPV-F</td>
<td>39.3</td>
<td>58.6</td>
<td>1.49</td>
<td>0.95–3.33</td>
<td>0.07</td>
</tr>
<tr>
<td>LukPV-S</td>
<td>129.0</td>
<td>228.3</td>
<td>1.77</td>
<td>1.12–2.81</td>
<td>0.01</td>
</tr>
<tr>
<td>LukD</td>
<td>25.0</td>
<td>67.4</td>
<td>2.69</td>
<td>1.28–5.62</td>
<td>0.01</td>
</tr>
<tr>
<td>LukE</td>
<td>19.9</td>
<td>30.0</td>
<td>1.51</td>
<td>0.89–2.52</td>
<td>0.126</td>
</tr>
<tr>
<td>HlgA</td>
<td>151.4</td>
<td>324.0</td>
<td>2.14</td>
<td>1.53–4.17</td>
<td>0.001</td>
</tr>
<tr>
<td>HlgB</td>
<td>32.8</td>
<td>41.6</td>
<td>1.27</td>
<td>0.80–2.11</td>
<td>0.17</td>
</tr>
<tr>
<td>HlgC</td>
<td>25.0</td>
<td>30.8</td>
<td>1.23</td>
<td>0.73–1.98</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*Acute-phase sera for which a convalescent pair was not obtained were excluded from this portion of the analysis.

*Paired t test used to determine statistical significance of the rise in acute-phase to convalescent-phase titer for each antigen.
Convalescent-phase sera neutralized LukAB-mediated toxicity in 1238 MSSA disease, and the difference was statistically significant for MSSA. Following invasive disease, the GMT for every antigen increase in titer against each subunit of PVL.

Samples were run in duplicate, independently on separate days, and a third run was performed if the first two titer values differed by one dilution. For LukAB, the GMT was 252.1 for LukA, 51.4 for LukB, 146.9 for Hla, 23.8 for V8 protease, 35.2 for LukPV-F, 59.0 for LukPV-S, 13.9 for LukD, 12.7 for LukE, 120.5 for HlgA, 30.4 for HlgB, and 19.2 for HlgC.

Neutralization of USA300 cytotoxicity. To further evaluate the breadth of neutralizing activity for the sera from the pediatric cohort, we tested the sera in an ex vivo infection assay where primary PMNs where challenged with a USA300 strain in the presence of different concentrations of acute-phase, convalescent-phase, and healthy control sera. Similar to the experiment using Newmann culture filtrates, the acute-phase and convalescent-phase sera were more potent at neutralizing USA300-mediated PMN membrane damage than healthy control sera (Fig. 6). In addition, we observed a modest but significant increase in protection by the convalescent-phase sera.

### DISCUSSION

The major findings of this study are that children with invasive S. aureus disease exhibit a high-titer, neutralizing antibody response to LukAB, and the rise in titer from acute disease to convalescence provides strong evidence of LukAB expression and production during invasive human disease. To our knowledge, this is the first description of the humoral immune response to LukAB, a recently described leukotoxin that is both abundantly produced by S. aureus and critical to S. aureus’ ability to subvert human PMNs (7, 12, 13, 24, 25). LukAB was ubiquitously present in clinical isolates from this cohort of children with invasive S. aureus disease, and the results of this study support the prominence of this toxin in the S. aureus proteome during human disease.

LukAB is increasingly recognized as a critical component of the S. aureus virulence repertoire devoted to evasion of human PMNs, although its exact role in vivo remains to be fully elucidated. Previous studies have shown that S. aureus, both MSSA and MRSA, kills human phagocytes in an LukAB-dependent manner (12, 13) and that disruption of lukAB markedly impairs the ability of S. aureus to avoid whole blood and PMN-mediated killing (7, 8, 12). Notably, deletion of lukAB in different S. aureus strains has a more prominent effect on PMN survival than the deletion of any other bicomponent leukotoxin (7, 8, 12). The prominent role of LukAB in the ability of S. aureus to target and kill PMNs, the first and often most critical line of defense to infection (26), is likely due to the increased expression of this toxin upon PMN encounter (8) and the fact that this toxin exploits CD11b as its receptor (7), a highly abundant protein on the surface of neutrophils.

This is one of the largest known studies involving paired (acute and convalescent) sera of children with invasive staphylococcal disease. The pediatric population confers several advantages in a study of the humoral response to S. aureus infection. First, extended carriage of S. aureus exerts an unknown effect on the serologic profile of specific antistaphylococcal antibodies. The pediatric serologic profile, lacking a lifetime of intermittent or persistent S. aureus nasal colonization, may more directly reflect the response to acute infection. Second, children with staphylococcal disease have uniquely defined phenotypes of invasive disease (e.g., acute hematogenous osteomyelitis and septic arthritis), allowing for more straightforward stratification of disease types. Third, the
relative lack of medical comorbidities and the known age-related dysregulation of humoral immune responses and reduced lymphocytes in the elderly (27, 28), potentially altering the immune response to S. aureus, are advantages to the study of the pediatric population.

Pediatric serologic studies to date have focused primarily on the response to PVL (29, 30), a bicomponent pore-forming toxin that targets neutrophils and is epidemiologically linked to epidemic strains of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) (19, 31). Hermos et al. measured antibody levels in 43 children with SSTI and 23 children with invasive disease (32). As in our study, they found that antibodies to PVL were detectable at low levels in healthy controls but were higher following disease. Neutralizing antibody to PVL did not appear to protect against SSTI, and the protective effects of antibodies against LukAB or other members of the bicomponent leukotoxin family have not been evaluated. A recent study by Fritz et al. measured the humoral response to alpha toxin and PVL in 56

![Graphs showing expression of LukA in clinical isolates from children with invasive S. aureus disease](image-url)
children with invasive \textit{S. aureus} disease, as well as 167 with noninvasive disease. The authors concluded that antibodies against alpha toxin were protective against future infection (24); however, antibody titers were not presented. In addition, the functional capacity of the antibodies to neutralize alpha toxin was not evaluated. Further, LukAB was not examined in this study, and future work in this area should focus on whether the generation of high-titer, neutralizing antibodies to LukAB alters subsequent disease risk.

Antibodies to \textit{S. aureus} antigens were detectable in healthy pediatric controls in this study, but at much lower titers than infected subjects. This is consistent with previous data suggesting that colonization may result in a low-level humoral response to staphylococcal antigens in children and adults (33–35), although the bicomponent leukotoxins were not reported in these previous studies. The role of colonization as an immunizing event remains unclear, as colonization appears to confer an increased risk of staphylococcal disease but may reduce mortality (36, 37). Antibody titers following noninvasive disease were not distinguishable from levels found in healthy controls. Since the majority of subjects in this study of hospitalized children had invasive disease, the study was not powered to detect statistically significant differences when comparing those with noninvasive disease to other groups.

Given reported differences in the severity of MRSA disease compared with MSSA disease (38), we compared the humoral response to infection stratified by methicillin resistance. Geometric mean titers against several exotoxins were significantly higher following MRSA disease than with MSSA disease. As the isolates were from a variety of genetic lineages, this difference is unlikely to be related to clonal relatedness of strains. This finding may be partially explained by recent data suggesting that the Agr regulatory system in epidemic CA-MRSA strains led to hypopressor expression of extracellular toxins and exoenzymes (39, 40). In children, invasive MRSA infection is associated with higher C-reactive protein levels and prolonged erythrocyte sedimentation rate, markers of a heightened inflammatory state (38, 41). The exuberant immune response stimulated by MRSA isolates compared to that by MSSA may have contributed to these serologic differences.

One caveat to our study is that culture-proven \textit{S. aureus} infection, which occurs at a time point that is not reached until several days into the disease process, was required for inclusion in the cohort. Thus, the acute-phase sera collected do not represent true baseline levels of antistaphylococcal antibodies. Analysis of the healthy control population, however, provides for comparison to the presumed baseline state. Additionally, convalescent-phase sera were collected 4 to 6 weeks after enrollment. Longitudinal data documenting persistence of this IgG response are lacking, and we plan to reassess the serologic profiles in these subjects at 1 year postinfection or longer. It should also be noted that the healthy control cohort was comprised of nonhospitalized subjects, a potential confounding factor, as the infected cases are all hospitalized patients with acute illness. It would not be expected, however, that hospitalization alone would alter the antistaphylococcal serologic profile, and thus the differences observed are likely to be a result of acute disease. Further, our sample size was not large enough to draw statistically significant conclusions regarding correlations between clinical disease severity and specific antibody titers, and this will be a focus of future work. Finally, \textit{in vitro} neutralization data are not meant to serve as a surrogate marker of protection \textit{in vivo} but rather as a measure of the functional activity of anti-LukAB antibodies. Work is ongoing to assess the \textit{in vivo} protective capacity of this antibody response.

The LukAB locus was present in all clinical isolates in this study of children with invasive \textit{S. aureus} disease and was a target of the natural host response to this pathogen. This immune recognition during human infection provides strong evidence that LukAB is produced \textit{in vivo}, suggesting a role in \textit{S. aureus} pathogenesis, as seen with other important staphylococcal antigens (42). Novel therapeutic and preventive options against \textit{S. aureus} are urgently needed, and recent reports suggest the benefits of a potential vaccine targeting staphylococcal exotoxins (24, 43), with the goal of preventing severe invasive disease rather than colonization or noninvasive illness (9). Given the functional antibody response produced against LukAB during invasive infection, further investigation of the role of this toxin in human disease is warranted.

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