Lower Antibody Levels to *Staphylococcus aureus* Exotoxins Are Associated With Sepsis in Hospitalized Adults With Invasive *S. aureus* Infections

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**Background.** *Staphylococcus aureus* has numerous virulence factors, including exotoxins that may increase the severity of infection. This study was aimed at assessing whether preexisting antibodies to *S. aureus* toxins are associated with a lower risk of sepsis in adults with *S. aureus* infection complicated by bacteremia.

**Methods.** We prospectively identified adults with *S. aureus* infection from 4 hospitals in Baltimore, MD, in 2009–2011. We obtained serum samples from prior to or at presentation of *S. aureus* bacteremia to measure total immunoglobulin G (IgG) and IgG antibody levels to 11 *S. aureus* exotoxins. Bacterial isolates were tested for the genes encoding *S. aureus* exotoxins using polymerase chain reaction (PCR).

**Results.** One hundred eligible subjects were included and 27 of them developed sepsis. When adjusted for total IgG levels and stratified for the presence of toxin in the infecting isolate as appropriate, the risk of sepsis was significantly lower in those patients with higher levels of IgG against α-hemolysin (Hla), δ-hemolysin (Hld), Panton Valentine leukocidin (PVl), staphylococcal enterotoxin C-1 (SEC-1), and phenol-soluble modulin α3 (PSM-α3).

**Conclusions.** Our results suggest that higher antibody levels against Hla, Hld, PVL, SEC-1, and PSM-α3 may protect against sepsis in patients with invasive *S. aureus* infections.

*Staphylococcus aureus* is a major human pathogen that causes community- and hospital-acquired infections. *S. aureus* most commonly causes skin and soft tissue infections with a range of severity, from uncomplicated boils to life-threatening infections complicated by bacteremia and sepsis. These infections have become progressively more difficult to treat because of multiple-antimicrobial resistance, such as methicillin resistance, among *S. aureus* isolates. The number of infections due to methicillin-resistant *S. aureus* (MRSA) has increased particularly in the community over the past decade [1]. *S. aureus* bacteremia leading to sepsis, often involving multiorgan failure, is associated with an attributable mortality of 20%–30%; the presence of sepsis increases the risk of death more than 2-fold [2, 3]. Although persistent nasal carriers of *S. aureus* are at increased risk of infection, the mortality rate among carriers is significantly lower compared to noncarriers [4]. This suggests that prior colonization is protective to a subsequent infection; perhaps due to adaptive immunity to the colonizing *S. aureus*.

*S. aureus* has a wide array of virulence factors, including a plethora of exotoxins that are aimed at
evading the host immune response as well as tissue destruction, and therefore can be associated with increased severity of infection. Some of these toxins, such as α-hemolysin (Hla) or Panton Valentine leukocidin (PVL), cause cytolysis of innate immune cells and erythrocytes, while others, such as superantigens, activate T-lymphocytes and lead to massive release of inflammatory cytokines, culminating in septic shock [5].

There is no US Food and Drug Administration–approved vaccine to prevent S. aureus infections, and efforts to develop a preventive vaccine have been unsuccessful [6]. Furthermore, patients with S. aureus infections often have recurrent infections with the same strain, and there is little evidence to suggest that lasting immunity exists. However, no one has explored whether a vaccine against S. aureus could reduce the severity of S. aureus infections. In particular, the potential benefit of including toxin-based vaccine components has not been completely explored. This study sought to identify potential targets of a toxin-based vaccine by assessing whether pre-existing antibodies to S. aureus toxins are associated with a lower risk of severity of infection, specifically sepsis, in adults with an S. aureus infection complicated by bacteremia.

METHODS

Study Design and Selection of Patient Population

This was a cohort study of adult patients ≥18 years with S. aureus infections complicated by bacteremia hospitalized at the University of Maryland (UM) Medical Center, the VA Maryland Health Care System, University Specialty Hospital, and Maryland General Hospital in Baltimore, MD, from July 2009 to May 2011. Potential study subjects were identified prospectively through microbiological data, when patients’ blood cultures first grew S. aureus. Serum samples from potential study subjects were collected prior to or at the time of their bacteremia (specifically, serum samples collected 0–3 days before the day the positive blood culture was drawn) to measure immunoglobulin G (IgG) antibody levels. This was possible because serum samples were routinely banked at 4°C for at least 3–7 days at each participating hospital. Medical records of potential study subjects were reviewed for eligibility. Patients were excluded if they were treated as outpatients; had polymicrobial infection; had symptoms of infection for greater than 4 days before the blood culture was drawn; had anticancer chemotherapy or radiation therapy within the preceding 36 months; had an active neoplastic disease or a history of any hematologic malignancy; had long-term use of oral steroids, parenteral steroids, or high-dose inhaled steroids in the preceding 6 months; or had a history of receiving immunoglobulin or other blood products within the 3 months preceding the first positive blood culture. The study was approved by the UM Baltimore Institutional Review Board and was granted a waiver of informed consent.

Data Collection and Study Variables

The exposure variable was serum IgG antibody level to 11 known staphylococcal exotoxins, and the outcome variable was sepsis within 3 days of bacteremia defined as severe sepsis or septic shock following the American College of Chest Physicians and Society for Critical Care Medicine definition [7]. Other variables shown in Table 1 were abstracted from the medical record.

Serological Measurements

Serum antibody to 10 known staphylococcal exotoxins, namely staphylococcal enterotoxins A (SEA), B (SEB), C-1 (SEC-1), D (SED), K (SEK), toxic shock syndrome toxin-1 (TSST-1), Hla, δ-hemolysin (Hld), and PVL components (LukS-PV and LukF-PV), was measured using an electrochemiluminescence (ECL)–based multiplex immunoassay on an MSD technology platform (Meso Scale Discovery, Gaithersburg, MD). The ECL method is a high-throughput, dynamic, multiarray system that uses a highly sensitive microplate reader equipped with camera and telecentric lenses that allowed for the simultaneous measurement of 10 antigen-specific antibodies from only 25 µL of serum. Antibody titers were determined using standard curves. Internal positive and negative controls were used to assess for inter- and intra-assay variability. The standards were specific human serum samples (identified through screening of multiple healthy individual serum samples) precharacterized on enzyme-linked immunosorbent assay (ELISA) plates coated with purified toxins. Full dilution curves were run for each standard and toxin, and the inflection point of 4-parameter logistic (4-PL) curves (half-maximal effective concentration) was assigned as the titer of the standard for the respective toxin. Using these annotated titers, standard curves were established in the multiplex ECL system and used for determination of antibody levels in patients’ serum samples. Antibody titers against phenol-soluble modulin α3 (PSM-α3) were determined on ELISA plates coated with 100 ng of the peptide. The plates were blocked with 4% milk in phosphate-buffered saline followed by the addition of 1:1000 diluted serum samples. Detection was performed by horseradish peroxidase enzyme-conjugated mouse antihuman antibody (Southern Biotech) and 3,3′,5,5′-tetramethylbenzidine substrate at optical density at 650 nm (OD650).

Total human IgG was determined in 1:50 000 diluted serum samples by using MULTI-ARRAY 96-well high-bind custom protein-A coated plates (high capacity) (Meso Scale Discovery). Total human IgG (Sigma-Aldrich/Fluka, St Louis, MO) over the concentration range of 10 µg/mL to 2 ng/mL was used to make the standard curve. Sulfo-tagged antihuman IgG was used as the detecting antibody and the signals were read
by MSD plate reader (Sector Imager 2400). Data were then analyzed by MSD workbench software provided by the manufacturer.

**Alpha-Toxin Neutralization Titer**

Alpha-toxin neutralization titers were determined based on neutralization of hemolysis of 2% rabbit red blood cells (RBCs) when dilutions of the serum samples were incubated with purified alpha toxin (100 ng/mL; List Biological Laboratories, Campbell, CA) at room temperature for 10 minutes, and then RBCs were added followed by incubation at 37°C for 30 minutes. After incubation, cells were pelleted, and the absorbance in the supernatant was determined in a microplate reader at OD416 nm. The neutralization titer 50% (NT50) was determined by plotting the OD416 nm against the dilutions using a 4-PI curve fit. NT50 is defined as the dilution of the serum that neutralizes alpha toxin by 50%.

**PVL Neutralization Assay**

PVL serum neutralization titers were based on cytotoxicity reduction in differentiated human promyelocytic leukemia cell line (HL-60) cells (ATCC, Manassas, VA). The HL-60 cells were propagated in Roswell Park Memorial Institute (RPMI) media supplemented with 15% fetal bovine serum (FBS) and 1.6% dimethyl sulfoxide. The differentiated neutrophil-like cells were harvested, suspended in phenol red–free RPMI/2% FBS, and used in the neutralization assay. Serum samples were diluted in V-bottom polypropylene 96-well plates, mixed with 400 ng PVL (1:1 LukS-PV and LukF-PV), and incubated for 30 minutes at 37°C. The cellular viability was evaluated after 16 hours of further incubation with 100 μg/mL 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.
(XTT; Sigma-Aldrich, St Louis, MO) and colorimetric measurement at OD₄₅₀ nm, from which NT₅₀ was determined for each sample.

**Microbiology and Toxin Gene/Hemolysin Presence**

Stocks of all *S. aureus* isolates were stored at −80°C in tryptic soy agar with 15% glycerol. Isolates were plated on sheep blood agar (BD Diagnostics, Sparks, MD) and confirmed as *S. aureus* by standard laboratory protocol. DNA extraction was performed using 250 μg/mL lysisostaphin and Prepman Ultra Sample Preparation Reagent ( Applied BioSystems, Carlsbad, CA). Polymerase chain reaction (PCR) analysis of 16S rDNA was used to verify *S. aureus* and presence of DNA in the sample. Toxin gene detection was performed by modifying multiplex and singleplex PCRs for SEA, SEB, SEC, SED, SEK, TSST-1, Hla, and LukF-PV [8]. Qualitative detection for Hla and Hld was performed on sheep blood agar SBA by the cross-streaking method as described previously [9].

**Statistical Analysis**

The association between the presence of sepsis and continuous variables was assessed using Student *t* test or the Wilcoxon rank sum test, as appropriate. The association between sepsis and categorical variables was assessed using the Pearson $\chi^2$ test or Fisher exact test, as appropriate. All antibody levels were log₁₀ transformed prior to analysis due to extreme non-normality. Two-way analyses of variance (ANOVAs) were performed to assess the association between the antibody level and sepsis, as well as the association between the antibody level and the presence of the antibody-specific toxin gene. The interaction between the antibody-specific toxin gene and sepsis was also included in the ANOVAs to determine if the association between antibody level and sepsis differed by presence or absence of the antibody-specific toxin gene. Logistic regressions were run separately for each antibody to examine the relationship between the antibody and presence of sepsis. Where appropriate, these regressions were stratified by the presence or absence of the antibody-specific toxin gene. All $P$ values provided are from 2-sided tests. All data analysis was performed using Stata 10 software (StataCorp, College Station, TX).

**RESULTS**

There were 422 patients with *S. aureus* bacteremia during the study period (Figure 1). Of these, serum was available for 227 patients (54%) and medical records were available for 225 of these patients. Of the 225 patients, more than half were not eligible due to the reasons shown in Figure 1. The most common reason for exclusion was immune suppression. After exclusion of ineligible patients, the study population consisted of 100 eligible patients with *S. aureus* bacteremia.

Patient characteristics were as follows: the mean age of the patients was 55 years (SD, 16 years), 54% were male, and 71% were African Americans. The mean Charlson comorbidity index was 3.7 (SD, 2.8), 24% had prior history of *S. aureus* infection, 28% were dialysis dependent, 11% had endocarditis, 58% had a nosocomial infection, and 63% had primary bacteremia. Of the 100 eligible patients, 27 had sepsis. The associations between patient characteristics and sepsis are displayed in Table 1. Older age, higher comorbidity as indicated by Charlson comorbidity score, no prior history of *S. aureus* infection, and primary bacteremia were associated ($P < .10$) with sepsis.

![Figure 1. Derivation of study population.](image-url)
The association between IgG antibody level to the 11 \textit{S. aureus} exotoxins and sepsis are displayed in Table 2. Patients with sepsis had significantly lower antibody levels against most of the toxins: Hla (\(P < .01\)), Hld (\(P < .01\)), LukF-PV (\(P = .02\)), LukS-PV (\(P = .01\)), SEA (\(P = .01\)), and SED (\(P = .01\)) than patients without sepsis. Patients with sepsis had significantly lower antibody levels against PSM-\(\alpha_3\) than patients without sepsis (0.13 vs 0.20; \(P = .01\), Wilcoxon). The total IgG level was also significantly lower in patients with sepsis (7.67 vs 9.05; \(P = .02\), Wilcoxon).

Among the 100 \textit{S. aureus} isolates, 53% had the PVL gene; 41%, SEK; 13%, SED; 11%, SEB; 7%, SEA; 4%, SEC; and 1%, TSST-1. Sixty-three percent of isolates exhibited, ex vivo, Hla activity and 80%, Hld activity when tested in sheep blood agar assay. Ex vivo hemolytic activity does not necessarily reflect the ability of the strain to produce such activities in infected individuals. We examined whether the presence of a toxin gene or hemolysin activity in the \textit{S. aureus} isolate modified the effect of the association between antibody level and sepsis for Hla activity, Hld activity, and presence of genes for SEB, SED, SEK, and PVL (Figure 2A and 2B). The association between antibody level and sepsis did not differ significantly by the presence of SED (\(P = .58\), 2-way ANOVA), SEK (\(P = .19\), 2-way ANOVA), Hla activity (\(P = .46\), 2-way ANOVA), or Hld activity (\(P = .48\), 2-way ANOVA). However, the association between antibody level and sepsis differed significantly by the presence of SEB (\(P = .02\), 2-way ANOVA), LukS-PV (\(P = .04\), 2-way ANOVA), or LukF-PV (\(P = .04\), 2-way ANOVA). This indicates that among patients whose infecting \textit{S. aureus} isolate have the PVL or PVL toxin genes, those with sepsis have lower antibody levels to the respective toxins than those without sepsis. This difference was not seen among patients whose infecting \textit{S. aureus} isolate did not have these toxin genes. Similarly, the association of toxin-neutralization activity against PVL (PVL-TNA; \(P < .01\), 2-way ANOVA) differed significantly by the presence of PVL genes.

Finally, the association between IgG antibody levels against specific exotoxins and sepsis was then evaluated, adjusting for total IgG and stratifying for the presence of toxin in the infecting isolate (see Table 3). The protective effect of antibodies against SEA, SED, and TSST was no longer statistically significant after controlling for total IgG. However, patients with higher antibody levels against Hla, Hld, PVLs, SEC-1, and PSM-\(\alpha_3\) had a significantly lower risk of sepsis before and after adjusting for total IgG level. Patients with higher antibody levels against LukF-PV, LukS-PV, and PVL-TNA had a significantly lower risk of sepsis before and after adjusting for total IgG level when the infection was caused by a PVL-positive \textit{S. aureus}, but not when the infection was caused by a PVL-negative \textit{S. aureus}. Because the number of patients whose infecting isolate had the SEB gene was low (\(n = 11\)), we were unable to adjust for total IgG among those patients. The study results were similar when patients with endocarditis or patients on dialysis were excluded (data not shown).

**DISCUSSION**

Our objective was to assess whether preexisting antibodies to specific \textit{S. aureus} toxins are associated with a lower risk of sepsis in adults with \textit{S. aureus} infection complicated by bacteremia. This study provides data that support the development of a multivalent toxin-based \textit{S. aureus} vaccine for the reduction of severity of invasive \textit{S. aureus} infection, rather than the prevention of infection. Several previous studies showed a correlation between the toxin produced by the infecting strain and the antibody response to the infection \cite{10, 11} but did not correlate antibody levels with clinical outcomes. Our results suggest that higher antibody levels against Hla, Hld, PVL, SEC-1, and PSM-\(\alpha_3\) may protect against sepsis in patients with invasive \textit{S. aureus} infections. Alternatively, higher antibody levels may be a marker of patients less likely to develop sepsis. To control for this, we adjusted our results for total IgG levels. We also assessed if the association was specific to whether the infecting isolate had the exotoxin genes of

**Table 2. Association Between IgG Antibody Level to 11 \textit{S. aureus} Exotoxins and Sepsis in Immunocompetent, Hospitalized Adults With Less Than 4 Days of Symptoms at the Time of \textit{S. aureus} Bacteremia**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Sepsis Median (IQR)</th>
<th>No Sepsis Median (IQR)</th>
<th>(P) value(^{ab})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hla</td>
<td>3.11 (2.68–3.35)</td>
<td>3.46 (3.15–3.83)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Hla TNA (NT(_{50}))(^f)</td>
<td>1.45 (1.02–1.77)</td>
<td>1.77 (1.43–2.02)</td>
<td>.01</td>
</tr>
<tr>
<td>Hld</td>
<td>1.24 (0.91–1.48)</td>
<td>1.73 (1.18–2.01)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>LukF-PV</td>
<td>1.94 (1.59–2.65)</td>
<td>2.75 (1.94–3.35)</td>
<td>.02</td>
</tr>
<tr>
<td>LukS-PV</td>
<td>2.34 (2.03–3.05)</td>
<td>2.98 (2.54–3.74)</td>
<td>.01</td>
</tr>
<tr>
<td>PVL-TNA</td>
<td>2.08 (1.57–2.98)</td>
<td>2.44 (1.78–3.08)</td>
<td>.17</td>
</tr>
<tr>
<td>SEA</td>
<td>1.98 (1.39–2.36)</td>
<td>2.32 (1.89–2.59)</td>
<td>.01</td>
</tr>
<tr>
<td>SEB</td>
<td>2.89 (2.32–3.35)</td>
<td>2.86 (2.46–3.34)</td>
<td>.49</td>
</tr>
<tr>
<td>SEC-1</td>
<td>2.64 (2.23–2.90)</td>
<td>2.89 (2.55–3.29)</td>
<td>.01</td>
</tr>
<tr>
<td>SED</td>
<td>0.86 (0.55–1.38)</td>
<td>1.31 (1.02–1.70)</td>
<td>.01</td>
</tr>
<tr>
<td>SEK</td>
<td>0.78 (0.46–1.17)</td>
<td>1.02 (0.60–1.33)</td>
<td>.13</td>
</tr>
<tr>
<td>TSST-1</td>
<td>2.52 (1.83–2.91)</td>
<td>2.64 (2.33–2.97)</td>
<td>.12</td>
</tr>
</tbody>
</table>

Abbreviations: Hla, \(\alpha\)-hemolysin; Hld, \(\delta\)-hemolysin; IQR, interquartile range; NT\(_{50}\), 50% toxin neutralization titer; PVL, Panton-Valentine leukocidin; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEC-1, staphylococcal enterotoxin C-1; SED, staphylococcal enterotoxin D; SEK, staphylococcal enterotoxin K; TNA, toxin neutralization assay; TSST-1, toxic shock syndrome toxin-1.

\(^a\) All antibody levels are log\(_{10}\) transformed.

\(^b\) \(P\) value from Wilcoxon test.

\(^c\) NT\(_{50}\) was carried out by TNA in 2% rabbit red blood cells. High- and low-titer human serum samples were used as standard controls for these assays.
interest, and we found that protection against sepsis may be specific to isolates expressing SEB and PVL.

Our results are consistent with previous studies of humoral responses with prior *S. aureus* colonization. Verkaik and colleagues [12] showed, in 57 colonized children, that IgG and immunoglobulin A levels for a number of staphylococcal proteins were higher than in noncolonized children despite extensive interindividual variability. Higher antibody levels to TSST-1, SEA, and clumping factors A and B have been reported in persistent carriers when compared to noncarriers [13]. One study demonstrated that *S. aureus* carriers had antibodies that neutralized superantigens for their colonizing strain [8]. In our study, in addition to toxin-specific antibody, a history of past *S. aureus* infection was also found to be protective against sepsis. These data provide biologic plausibility to our argument that toxin-specific antibodies may protect against the severity of infection.

Many of the *S. aureus* toxins that were identified in this study are known lytic virulence factors, which can individually determine the severity of disease outcome. Hla is a prolytic pore-forming toxin [14,15] for which the gene (*hla*) is chromosomal and expressed by most *S. aureus* isolates [14,16] and against which passive [17] and active antibodies [18,19] can modulate disease outcome in animal models of infection. The similarly prolytic [20] δ-toxin gene is believed to be present in all *S. aureus* isolates [21,22] and may act in strong synergism with both α- and β-toxins [23] to promote cellular lysis. PSM peptides, PSM-α group (PSM-α1, PSM-α2, PSM-α3, and PSM-α4) and PSM-β, have been identified as key virulence factors of emerging community-associated MRSA and other highly pathogenic *S. aureus* strains [24–27]. PVL-expressing strains, which cause severe or lethal skin, bone, and lung infections in animal models [28–31], are clinically associated with invasive, severe infections, particularly those complicated with life-threatening pneumonia [32–34]. The separately secreted PVL components, LukS-PV and LukF-PV, form a pore-forming octameric complex [35], which causes cell lysis and may also lead to impairment of the innate and humoral immune responses. Besides PVL, other bicomponent toxins have been characterized in *S. aureus*, including multiple S and F γ-hemolysins (Hlg) and leukotoxin E and D components. A recent report suggests a role for Hlg in survival of *S. aureus* in blood [36]. Due to high degree of sequence identity between PVL and Hlg components, it is possible that antibodies to PVL may also neutralize Hlg and further contribute to reduced severity of the infection.

Meanwhile, *S. aureus* isolates can also contain pyrogenic toxin superantigen (PTSAg) genes, comprising the SE genes and TSST-1 gene; 80% of *S. aureus* harbor at least 1 PTSAg [37, 38]. These powerful mitogenic toxins have been implicated in the pathogenesis of several acute or chronic human disease states [39, 40]. In our study, SEC-1 antibody was

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**Figure 2.** Association between IgG antibody level to specific *S. aureus* exotoxin and sepsis, stratified by the presence of hemolytic activity (A) or toxin gene (B) in the infecting *S. aureus* isolate. An * indicates that the *P* value of the ANOVA interaction term is ≤0.05. Abbreviations: IgG, immunoglobulin G; LukF-PV, Panton-Valentine leukocidin F; LukS-PV, Panton-Valentine leukocidin S; SEB, staphylococcal enterotoxin B; SED, staphylococcal enterotoxin D; SEK, staphylococcal enterotoxin K.
Table 3. Risk of Sepsis given IgG Level to *S. aureus* Exotoxins Before and After Adjusting for Total IgG Level in Immune Competent, Hospitalized Adults with Less than 4 Days of Symptoms at the Time of *S. aureus* Bacteremia (n = 100)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>OR( ^{a} ) Unadjusted</th>
<th>P value</th>
<th>OR Adjusted for Total IgG</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hla</td>
<td>0.25</td>
<td>&lt;.01</td>
<td>0.34</td>
<td>.03</td>
</tr>
<tr>
<td>Hla TNA (NT50)(^{b} )</td>
<td>0.50</td>
<td>.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hld</td>
<td>0.23</td>
<td>&lt;.01</td>
<td>0.28</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>SEA</td>
<td>0.36</td>
<td>.02</td>
<td>0.46</td>
<td>.08</td>
</tr>
<tr>
<td>SEB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>0.68</td>
<td>.21</td>
<td>0.76</td>
<td>.38</td>
</tr>
<tr>
<td>Subjects with SEB gene</td>
<td>0.11</td>
<td>.15</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Subjects without SEB gene</td>
<td>0.86</td>
<td>.64</td>
<td>0.95</td>
<td>.88</td>
</tr>
<tr>
<td>SEC-1</td>
<td>0.33</td>
<td>.01</td>
<td>0.40</td>
<td>.04</td>
</tr>
<tr>
<td>SED</td>
<td>0.40</td>
<td>.03</td>
<td>0.50</td>
<td>.12</td>
</tr>
<tr>
<td>SEK</td>
<td>0.59</td>
<td>.21</td>
<td>0.77</td>
<td>.52</td>
</tr>
<tr>
<td>TSST</td>
<td>0.48</td>
<td>.05</td>
<td>0.60</td>
<td>.20</td>
</tr>
<tr>
<td>LukF-PV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>0.57</td>
<td>.03</td>
<td>0.64</td>
<td>.11</td>
</tr>
<tr>
<td>Subjects with PVL gene</td>
<td>0.39</td>
<td>.01</td>
<td>0.44</td>
<td>.04</td>
</tr>
<tr>
<td>Subjects without PVL gene</td>
<td>0.97</td>
<td>.95</td>
<td>0.99</td>
<td>.99</td>
</tr>
<tr>
<td>LukS-PV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>0.48</td>
<td>.02</td>
<td>0.55</td>
<td>.06</td>
</tr>
<tr>
<td>Subjects with PVL gene</td>
<td>0.26</td>
<td>&lt;.01</td>
<td>0.30</td>
<td>.02</td>
</tr>
<tr>
<td>Subjects without PVL gene</td>
<td>0.86</td>
<td>.73</td>
<td>0.87</td>
<td>.75</td>
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<tr>
<td>PVL-TNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td>0.72</td>
<td>.24</td>
<td>0.82</td>
<td>.50</td>
</tr>
<tr>
<td>Subjects with PVL gene</td>
<td>0.26</td>
<td>&lt;.01</td>
<td>0.29</td>
<td>.01</td>
</tr>
<tr>
<td>Subjects without PVL gene</td>
<td>1.77</td>
<td>.16</td>
<td>1.74</td>
<td>.19</td>
</tr>
<tr>
<td>PSM-α3(^{c} )</td>
<td>0.17</td>
<td>.02</td>
<td>0.23</td>
<td>.04</td>
</tr>
</tbody>
</table>

Abbreviations: IgG, immunoglobulin G; NT50, 50% toxin neutralization titer; OR, odds ratio; PSM-α3, phenol-soluble modulin; PVL, Panton-Valentine leukocidin; SEA, staphylococcal enterotoxin A; SEB, staphylococcal enterotoxin B; SEC-1, staphylococcal enterotoxin C-1; SED, staphylococcal enterotoxin D; SEK, staphylococcal enterotoxin K; TNA, toxin neutralization assay; TSST-1, toxic shock syndrome toxin-1.

\(^{a}\) All toxin-specific IgG variables were log_{10} transformed prior to analysis. Toxin-specific IgG ORs represent the average change in odds of sepsis from each log_{10} unit increase in toxin-specific IgG level.

\(^{b}\) NT50 was carried out by TNA in 2% rabbit red blood cells. High- and low-titer human serum samples were used as standard controls for these assays.

\(^{c}\) PSM-α3 antibody levels were measured in optical density at 650 nm by traditional enzyme-linked immunosorbent assay in 1:1000 diluted samples.

statistically significant before and after adjusting for total IgG, whereas for other superantigens, there was a trend toward protection, but significance was not as pronounced. This could have been due to our low sample size or because anti–SEC-1 antibody cross-reacts with SEB and SEA [41–43], which suggests that these antibodies may have broad-spectrum neutralizing activity against heterologous superantigen-expressing strains. The importance of antibodies against superantigens is potentially highlighted in that, though the number of patients infected with SEB-producing strains was low (n = 11), only 2 developed sepsis and the geometric mean antibody titer of anti-SEB in these patients was more than 1 log lower than in patients with SEB-producing strains who did not develop sepsis.

The major limitation of our study is that it is observational. We detected an association, but cannot prove that the relationship is causal. However, our study provides justification for a subsequent prospective or interventional study. Although this study is limited by a small sample size, we were able to identify statistically significant differences among the targeted toxins—therefore, the effect of antibodies against these toxins is potentially large and further justifies our therapeutic rationale. Our patient population was geographically limited to the Baltimore area, which could potentially limit external generalizability. And finally, since these superantigens are variably present in *S. aureus* isolates, we were limited in our ability to assess the less common superantigens.

In conclusion, our results suggest that higher preexisting antibody levels against Hla, Hld, PVL, SEC-1, and PSM-α3 may protect against sepsis in patients who develop invasive *S. aureus* infection. In addition, we found that higher preexisting antibody against PVL and SEB were protective against sepsis, only when PVL or SEB genes were present in the infecting isolate. Our findings underscore the potential importance of antibodies to superantigens such as SEB and SEC-1 in protection against sepsis. Over 19 superantigens are variably expressed in different *S. aureus* clinical isolates, posing a challenge for the inclusion of all superantigens in a multivalent *S. aureus* vaccine. However, it may be feasible to include only a few of the most frequently expressed toxins. Taken together, our results support our therapeutic rationale for passive or active immunization with a multivalent vaccine, which elicits high antibody levels against selected toxins for the reduction of severity of invasive *S. aureus* infections.

Notes

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