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MAJOR ARTICLE

Vaccination With Detoxified Leukocidin AB Reduces Bacterial Load in a *Staphylococcus aureus* Minipig Deep Surgical Wound Infection Model

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Vaccines against *Staphylococcus aureus* have eluded researchers for >3 decades while the burden of staphylococcal diseases has increased. Early vaccine attempts mainly used rodents to characterize preclinical efficacy, and all subsequently failed in human clinical efficacy trials. More recently, leukocidin AB (LukAB) has gained interest as a vaccine antigen. We developed a minipig deep surgical wound infection model offering 3 independent efficacy readouts: bacterial load at the superficial and at the deep-seated surgical site, and dissemination of bacteria. Due to similarities with humans, minipigs are an attractive option to study novel vaccine candidates. With this model, we characterized the efficacy of a LukAB toxoid as vaccine candidate. Compared to control animals, a 3-log reduction of bacteria at the deep-seated surgical site was observed in LukAB-treated minipigs and dissemination of bacteria was dramatically reduced. Therefore, LukAB toxoids may be a useful addition to *S. aureus* vaccines and warrant further study.

Keywords. leukotoxin AB; minipig; surgical wound infection; *Staphylococcus aureus*; vaccine.

Staphylococcus aureus (SA) is a leading cause of hospital-acquired infections, such as surgical site infections, pneumonia, and sepsis, and is a common cause of community-acquired skin and soft tissue infections and bloodstream infections [1, 2]. There is a substantial unmet medical need for an effective vaccine to prevent serious SA infections, many of which are now multidrug resistant [2]. Although this has been a goal for decades, to date, no vaccine has shown efficacy in humans [3]. Single-component (Merck's V710 vaccine targeting iron-regulated surface determinant B), bivalent (Nabi Biopharmaceuticals' StaphVAX vaccine containing conjugated capsular polysaccharides [CPs] 5 and 8), and 4-component (Pfizer's CP5 and CP8 conjugates, clumping factor A, and manganese transport protein C) [4] vaccines all failed to demonstrate clinical efficacy [5]. Published theories on why these vaccines were not effective include their focus on generating opsonic antibodies and their reliance on preclinical mouse models of infection [5]. Staphylococcal manipulation of host immune responses is essential for pathogenesis and such bacterial immune evasion factors must be neutralized before opsonophagocytosis can occur [6]. There is also increasing evidence that protection against staphylococcal disease requires a balance of both cellular and humoral immunity against appropriate targets [5]. Mice are a poor model with which to study this balance, as laboratory mice often have low levels of exposure to SA [7] and, consequently, a very different level of immunological priming compared to humans. Furthermore, SA causes disease in multiple organ systems, and differences in virulence factors among strains may influence the clinical manifestations of the disease [8]. Often, to predict vaccine efficacy, multiple infection models in mice, rats, and/or rabbits are used to mimic different clinical manifestations. The SA strains used in these models are generally either laboratory- or mouse-adapted, and the model requires a high inoculum for a robust infection, resulting in models that do not resemble the disease progression in humans [9].

Dependence on rodent models particularly hinders development of vaccines to virulence factors of SA that are species-specific, such as the bicomponent pore-forming leukotoxins, many of which are being investigated as promising vaccine candidates [3, 10–12]. Leukocidin AB (LukAB) has been shown to be the primary toxin responsible for primary human polymorphonuclear leukocyte (PMN) cell death during tissue culture infection [13], and impairs function of and kills antigen presenting cells [14], thus potentially reducing the host defense and immunological memory needed to combat current and subsequent infections. Despite potent activity of LukAB toward

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primary human phagocytes, the higher affinity of the toxin to the human CD11b receptor compared to the murine receptor [13, 15] renders commonly used mouse models incompatible for studying LukAB.

Progress in the field of staphylococcal vaccines is likely to stall unless alternative animal challenge/protection models are developed that closely resemble human staphylococcal disease. In this study we describe an SA surgical wound infection model in Göttingen minipigs (Minipig surgical wound infection). Pigs are natural hosts for SA, and transmission of disease between pigs and humans has been documented [16–18]. Similarities between the immune systems of humans and pigs include a high percentage of circulating neutrophils, a lack of nitric oxide production following lipopolysaccharide stimulation, similarity of Toll-like receptors and dendritic cells, and a comparable response to endotoxin challenge [19]. Preexisting antibodies to various SA antigens are present in pigs, as seen in humans [20, 21]. The skin of pigs is structurally similar to human skin in terms of thickness, number of hair follicles, pigmentation, and collagen and lipid composition. Pigs have also been used extensively as a research model for wound healing, drug delivery, toxicology, and dermatological conditions [22, 23], and use of the minipig as a higher animal model for toxicology testing has gained regulatory acceptance [23–26]. Additionally, SA has been studied in several pig challenge models [27–30], including a swine model used to evaluate the treatment of SA surgical site infections [31]. To our knowledge, pig models have not been used to date to study deep-seated SA infections or to evaluate vaccine efficacy.

MATERIALS AND METHODS

Animals

Five- to 8-month-old male Göttingen minipigs were group-housed and maintained on a 12-hour light/dark cycle with access to water ad libitum. Blood was collected before prime and boost doses, and 3 and 8 days postinfection.

All animal procedures were approved by the Janssen Spring House Institutional Animal Care and Use Committee and conducted in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility in accordance with US Department of Agriculture Animal Welfare Regulations, the Guide for the Care and Use of Laboratory Animals [32, 33], and institutional policies.

Minipig Surgical Wound Model

Fasted minipigs were sedated, intubated, and placed under isoflurane anesthesia for the duration of the surgery. After sterile preparation of the skin, a skin incision was made to expose the muscle layer on the left thigh and a 5-mm bladeless trocar was advanced to the depth of the femur. Twenty microliters of inoculum (~6 $\log_{10}$ colony-forming units [CFU] SA) was injected into the wound via a 6-inch MILA spinal needle through the trocar and the muscle was closed with silk suture and the skin closed with absorbable Vicryl suture (Figure 1A).

Eight days later, following euthanasia, the skin was cleaned with 2% chlorohexidine solution and the surgical site skin and full thickness surgical site muscle (2 cm^2) was removed and cut into 3 sections (superficial, mid, and deep layers) and processed separately. The skin and spleen were aseptically removed and processed separately.

Bacterial Strains and Growth Conditions

Three clinical blood isolates of SA were used to characterize the minipig model: ST398 (OC 26263, methicillin-susceptible SA) was used for the LukAB minipig challenge studies (ST398 strains typically colonize pigs but can cause disease in humans [34–36]); ST5 (OC 26245, methicillin-resistant SA [MRSA]) and ST8 (OC26260, MRSA) were included because of their high global prevalence among healthcare-associated and community-acquired MRSA infections [37]. Strains were grown in Tryptic soy broth overnight prior to use.

For opsonophagocytic assays, the ST398 challenge strain expressing CP5 and MRSA ST30 clinical strain (BVSA00929) expressing CP8 were used. Strains were grown for 24 hours on Columbia salt agar to induce capsule expression.

Antigen Production and Formulation

Genetically detoxified LukAB (CC8 LukAB sequence where LukA had a deletion at the 10 C-terminal amino acid residues; LukABΔ10C toxoid) and wild-type LukAB CC8 toxin were expressed in SA and subsequently purified as described previously [38].

CP5 and CP8 were purified from SA clinical strains and conjugated to CRM197 through thioether chemistry (CP5/8-CRM).

HlaH35L was produced at GenScript. In brief, the sequence of SA Newman HlaH35L, cloned behind an N-terminal His-SUMO tag, was expressed in *Escherichia coli* BL21 by IPTG induction and affinity-purified from the supernatant of the whole cell lysate. After purification, the His-SUMO tag was removed.

Vaccination

Antigens were combined 1:1 (v/v) with $AS01_B$ adjuvant 30 minutes prior to vaccination. Minipigs were sedated (Telazol) and bled prior to being immunized (3 intramuscular injections separated by 3 weeks) with CP5/8-CRM and LukABΔ10C toxoid (100 μg) mixed 1:1 with $AS01_B$ adjuvant resulting in a one-half human adjuvant dose. CP5/8-CRM was compared to unadjuvanted formulation buffer (Tris-buffered saline, pH 7.4, 10% glycerol) and LukAB was compared to adjuvanted formulation buffer (separate studies). Surgery and infection occurred 3 weeks after the last boost.

Enzyme-Linked Immunosorbent Assay and Cytokine Analysis

To determine total immunoglobulin G (IgG) in serum, antigens were coated onto 96-well Maxisorp plates (Nunc) for minimum 1 hour, to maximum overnight, at 2°C–8°C. Plates were blocked with

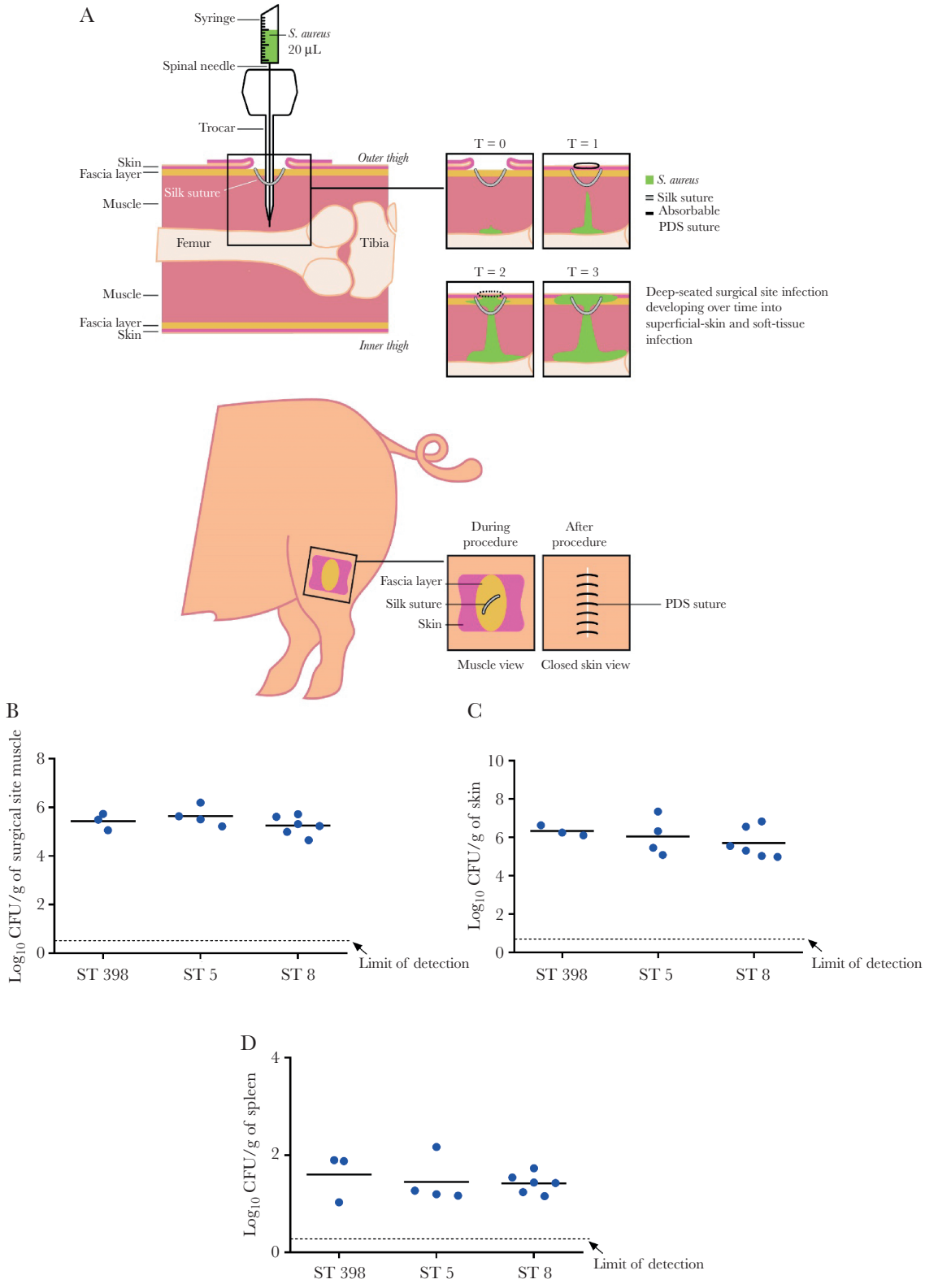

Figure 1. Bacterial growth of clinically relevant *Staphylococcus aureus* strains in the minipig. *A*, Minipig deep surgical wound infection model. Bacterial burden after 8 days of infection with sequence type (ST) 398, ST5, and ST8 strains in the surgical site muscle (*B*), skin (*C*), and spleen (*D*). Abbreviations: CFU, colony-forming units; PDS, polydioxanone suture (other absorbable may be substituted); ST, sequence type.

2.5%–5% (w/v) skimmed milk prior to washing and subsequent addition of serial dilution of serum. Secondary antibody (rabbit-antipig IgG horseradish peroxidase [HRP]) was added at 1:40 000 dilution (1:20 000 for LukAB). After further washing, 3,3′,5,5′-tetramethylbenzidine was added to detect the HRP. The reaction was stopped with 1 M sulfuric acid, and absorbance was read at 450 nm.

To measure total IgG against CP5 and CP8, CP5-biotin and CP8-biotin conjugates were used to facilitate plate coating.

Concentrations of 13 cytokines in plasma were determined using the MILLIPLEX MAP Porcine Cytokine/Chemokine Magnetic Bead Panel.

Opsonophagocytic Assay

HL-60 cells (American Type Culture Collection CCL-240) were differentiated into phagocytes by supplementing culture media with 0.8% dimethylformamide for 4–5 days. Bacteria were incubated with heat-inactivated sera and IgG/immunoglobulin M–depleted human sera (PelFreez) as a source of complement, prior to addition of differentiated HL-60 cells (HL-60:bacteria ratio 400:1). After 45 minutes, saponin was added to a final concentration of 1%. CFUs were counted after overnight incubation. The killing titer was calculated as the reciprocal of the highest serum dilution that gave 40% killing compared to a no-serum control.

Toxin Neutralization Assay

Toxin neutralization assays were performed with THP-1 monocytes, using a method adapted from Melehani et al [39]. THP-1 cells were added to 96-well plates at 1×10^5 cells/well, along with heat-inactivated minipig serum and LukAB CC8 toxin (final concentration 20 ng/mL). After 2 hours of incubation, lactate dehydrogenase was measured in supernatant using the CytoTox-ONE assay. Percentage cytotoxicity was calculated relative to a cell and toxin-only control. Half maximal inhibitory concentration titers were determined by 4PL curve fitting.

PMN Isolation and Cytotoxicity Assessment

Human and minipig PMNs were isolated from leukopaks as previously described [40]. PMNs were plated at 200 000 cells per well in 90 µL/well in 96-well plates. CC8 LukAB toxin from SA was diluted to test concentrations ranging from 20 µg/mL to 0.015 µg/mL and added at 10 µL/well. After 90 minutes of incubation, toxicity was measured by adding CellTiter 96 Aqueous One Solution (10 µL/well), incubating for a further 90 minutes, and reading absorbance at 492 nm.

Statistical Analysis

Groups were compared using a Tobit regression model to account for possible censoring for the analysis of the CFU response. For the analysis of the interleukin 6 (IL-6) response, groups were compared using an analysis of variance (ANOVA) model. The Tobit and ANOVA models both contained treatment group and study number as explanatory factors.

RESULTS

SA Clinical Isolates Produced a Robust Infection in Minipigs

Bacterial burden at the infection site deep in the thigh was similar at 8 days postinfection (6–7 $\log_{10}$ CFU/g tissue) regardless of challenge strain (ST398, ST5, or ST8) (Figure 1). At necropsy,

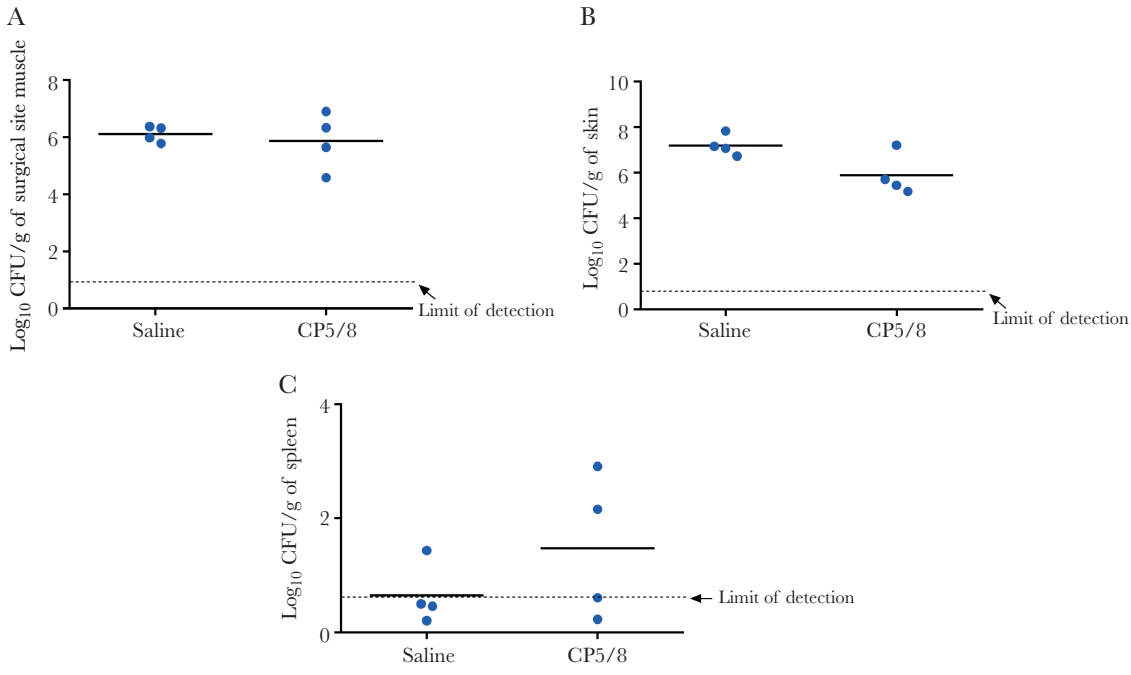

Figure 2. Effect of capsular polysaccharides 5 and 8 in the muscle, skin, and spleen of the minipig. Bacterial burden after 8 days of infection (sequence type 398) in the surgical site muscle (*A*), skin (*B*), and spleen (*C*). Abbreviations: CFU, colony-forming units; CP5/8, capsular polysaccharides 5 and 8.

there was purulent material found in all 3 muscle layers and under the skin, and bacteria disseminated from the infection site to the abdominal organs (Figure 1).

Immunization With CP5/8-CRM197 Failed to Protect Minipigs From Surgical Site Infection

To evaluate the minipig model as a tool to investigate vaccine-mediated protection, minipigs were immunized with a full human dose of CP5/8-CRM197, as previously described [4], in combination with adjuvant, on 3 occasions prior to challenge with the CP5-expressing ST398 strain. At necropsy, there were no significant differences in bacterial burden at the surgical site or in the spleen (indication of dissemination) of animals immunized with the CP-conjugate mix vs animals immunized with saline (Figure 2).

All vaccinated animals generated robust IgG titers after vaccination against CP5, CP8, and CRM197 (Figure 3), which were not associated with increases in opsonophagocytic assay (OPA) titers in vitro against either the $CP5^+$ ST398 challenge strain or against a $CP8^+$ strain (Figure 3). To confirm that each strain expresses capsule under the growth condition used for the assay, OPA was also performed with monoclonal antibodies against CP5 and CP8 (Supplementary Figure 1).

OPA and IgG titers against staphylococcal Hla in minipig sera were already positive at the beginning of the study and increased over time (Figure 3), which is consistent with natural colonization of pigs by SA [41]. IgG against Hla was further measured in serum samples from a larger number of minipigs ranging from 4 months to 18 months of age (Supplementary Figure 2). Antibodies against Hla increased with increasing age, indicating that exposure to SA and natural immunity are common in Göttingen minipigs.

LukAB Showed Comparable Cytotoxicity to Human and Minipig PMNs In Vitro

In contrast to rodent models, LukAB has been shown to bind pig CD11b to a similar level as human CD11b [15]. Here, we evaluated whether LukAB also shows comparable cytotoxicity toward minipig PMNs. When granulocytes isolated from minipig blood were challenged with wild-type LukAB toxin, they showed a similar susceptibility to the toxin as human PMNs (Figure 4). By contrast, LukAB exhibits markedly reduced cytotoxicity on

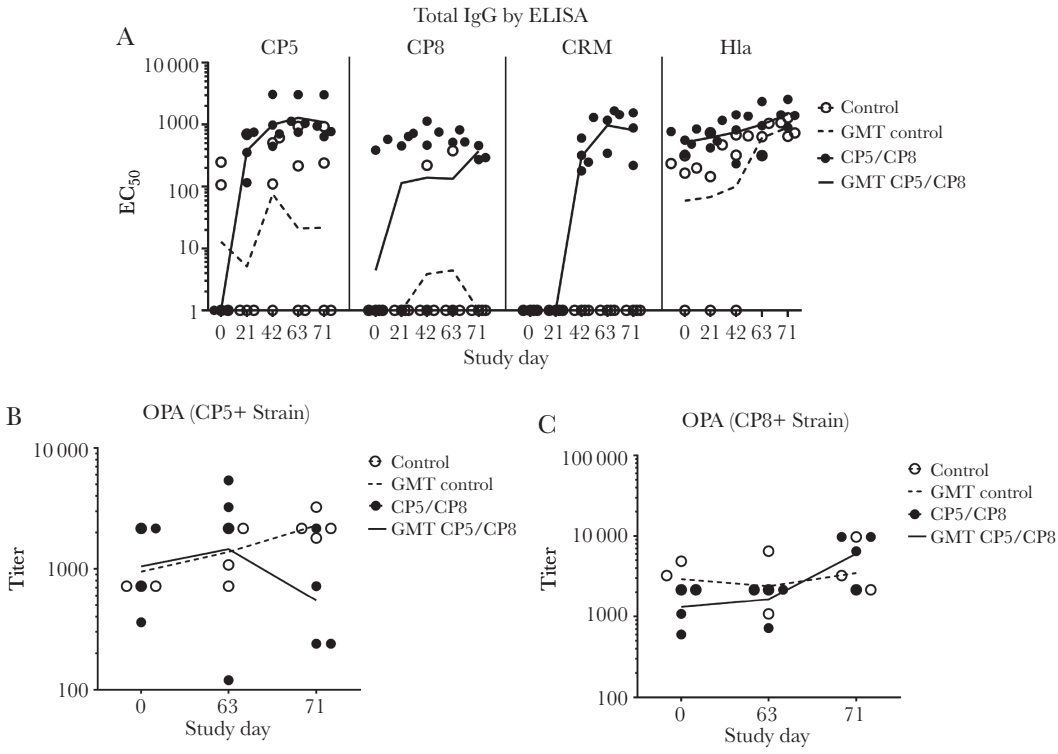

Figure 3. Humoral immunity generated after immunization with capsular polysaccharide 5 (CP5)–CRM and capsular polysaccharide 8 (CP8)–CRM conjugates. Total immunoglobulin G was measured by enzyme-linked immunosorbent assay (ELISA) (*A*) against CP5 and CP8 (in both cases, polysaccharides were conjugated to biotin to facilitate plate coating), CRM197, and Hla. Opsonophagocytic activity (OPA) of sera was measured using differentiated HL-60 cells and human complement against a CP5-expressing sequence type (ST) 398 clinical isolate (*B*) and a CP8-expressing ST30 clinical isolate (*C*), with OPA titers reported as the reciprocal of the serum dilution giving 40% killing compared to a no-serum control. In all cases, results are shown as half maximal effective concentration (EC_{50}) values (ELISA) or OPA titers per animal per timepoint (n = 4 animals per group), with a line at the geometric mean titer (GMT) per group. Open circles and dashed lines indicate animals in the adjuvant-control group, while closed circles and complete lines indicate animals immunized with the CP5/8-CRM197 vaccine. Study days are measured from the day of the first vaccination, ie, day 0, 21, and 42 = dose 1, 2, and 3, respectively; day 63 = challenge, day 71 = necropsy.

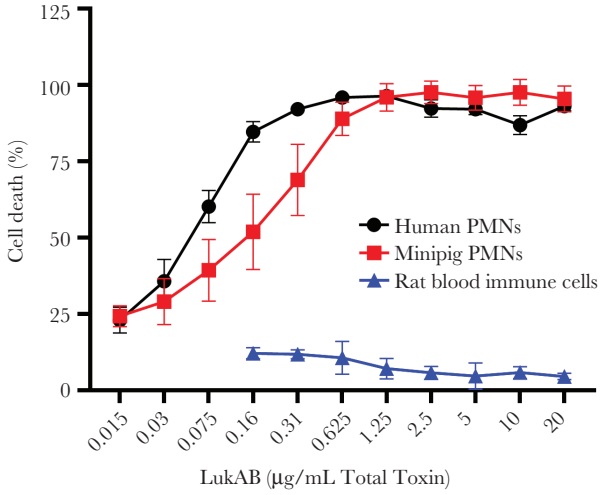

Figure 4. Toxicity of leukocidin AB (LukAB) toward human and minipig polymorphonuclear leukocytes (PMNs). Intoxication of freshly isolated human or minipig PMNs and rat blood immune cells with the indicated doses of LukAB for 1.5 hours. Cell viability was evaluated with the metabolic dye CellTiter, and percentage of cell death was determined by normalizing to cells treated with TritonX-100 set at 100% cell death.

rat immune cells (used to estimate mouse) and PMNs isolated from mice and rabbits [42].

LukAB Vaccination Protected Minipigs From Surgical Site Infection

To explore whether the minipig surgical wound infection model could show any protection afforded by vaccination with LukAB, minipigs were vaccinated with 3 doses of a detoxified LukAB protein lacking the CD11b-targeting domain (LukAB CC8Δ10C, [38]), or an adjuvant control, prior to infection with the ST398 challenge strain. The experiment was performed across 2 separate studies, each containing 3 LukAB toxoid–vaccinated animals and 3 adjuvant control animals.

Sera from all minipigs contained measurable IgG against the wild-type LukAB CC8 toxin as well as preexisting antibodies to Hla at day 0 (Figure 5). In the control group, anti-LukAB IgG titers increased between challenge and necropsy, suggesting that not only is LukAB expressed by the SA strains that naturally colonize minipigs, but also that LukAB is actively expressed during infection in this model.

LukAB vaccination resulted in increased anti-LukAB IgG titers compared to the control group, and these correlated with an increased capacity of the sera to neutralize cytotoxicity of the wild-type toxin against THP-1 cells in vitro (Figure 5). This difference was largest at day 63 (day of challenge). By day 71 (necropsy), anti-LukAB IgG and toxin neutralization by sera were similar between the LukAB toxoid–vaccinated and the control animals, due to the boosting effect of LukAB exposure during challenge in the adjuvant group. The same boosting effect during challenge was not seen in the LukAB toxoid–vaccinated group.

At necropsy, animals vaccinated with LukAB toxoid had significantly ($P < .0001$) lower CFU counts at the surgical site muscle compared to the adjuvant group (Figure 6A), with the greatest difference observed in the deepest muscle depth ($P < .0001$), immediately above the femur (Figure 6B), with CFU counts in the superficial muscle (below the skin) being closer to those measured in the adjuvant control group (Supplementary Figure 3). Bacterial burden in the skin was similar between the groups; however, protection at the surgical site in the LukAB vaccinated group was clearly visible as a reduction in purulent material throughout the muscle (Figures 6 and 7). Furthermore, animals vaccinated with LukAB toxoid had significantly lower CFU counts in the spleen than control animals (5/6 at or below the limit of quantification vs 1/6, respectively; Figure 6D), indicating that immunization provided protection both at the surgical site and against dissemination of the bacteria from the surgical site.

As an additional marker of a more systemic response to infection, levels of 13 cytokines were measured in plasma from the minipigs prior to challenge, at days 1, 2, and 3 after challenge, and at necropsy. In control animals, IL-6, a key marker for systemic inflammation during infection, increased sharply within 24 hours after infection (Figure 5; other cytokines included in Supplementary Table 1), before returning to baseline by day 8 postinfection. In general, IL-6 concentrations in plasma of LukAB toxoid–vaccinated animals 24 hours after challenge were lower than those measured in control animals; however, due to low group sizes and high variability among animals, this did not achieve significance ($P = .06$).

This increase in IL-6 was concordant with the rise in body temperature immediately following infection and lasting 48–72 hours; however, there was no difference in body temperature between the LukAB toxoid–immunized animals or the animals treated with adjuvant control.

DISCUSSION

To address the need for a preclinical model of SA infection that is more representative of the human state than rodents, we have developed an SA deep-seated surgical wound infection model in Göttingen minipigs. Pigs share many characteristics with humans that make them suitable for such a model, for example, similarities in the skin [22] and the immune system [19]. Pigs have been used previously for a variety of SA challenge models, including skin, osteomyelitis, and pyemia [27, 43, 44], demonstrating the versatility of this species, but have not to our knowledge been used as a model for deep-seated surgical wound infection. Pigs are a natural reservoir for SA [45]. Consequently, it is possible to use human clinical isolates in the minipig challenge model. In our model, we studied 3 human clinical isolates: ST5 (USA100), ST8 (USA300), and ST398. In all cases, we were able to induce a reproducible, high bacterial burden at the deep-seated infection site as well as at superficial

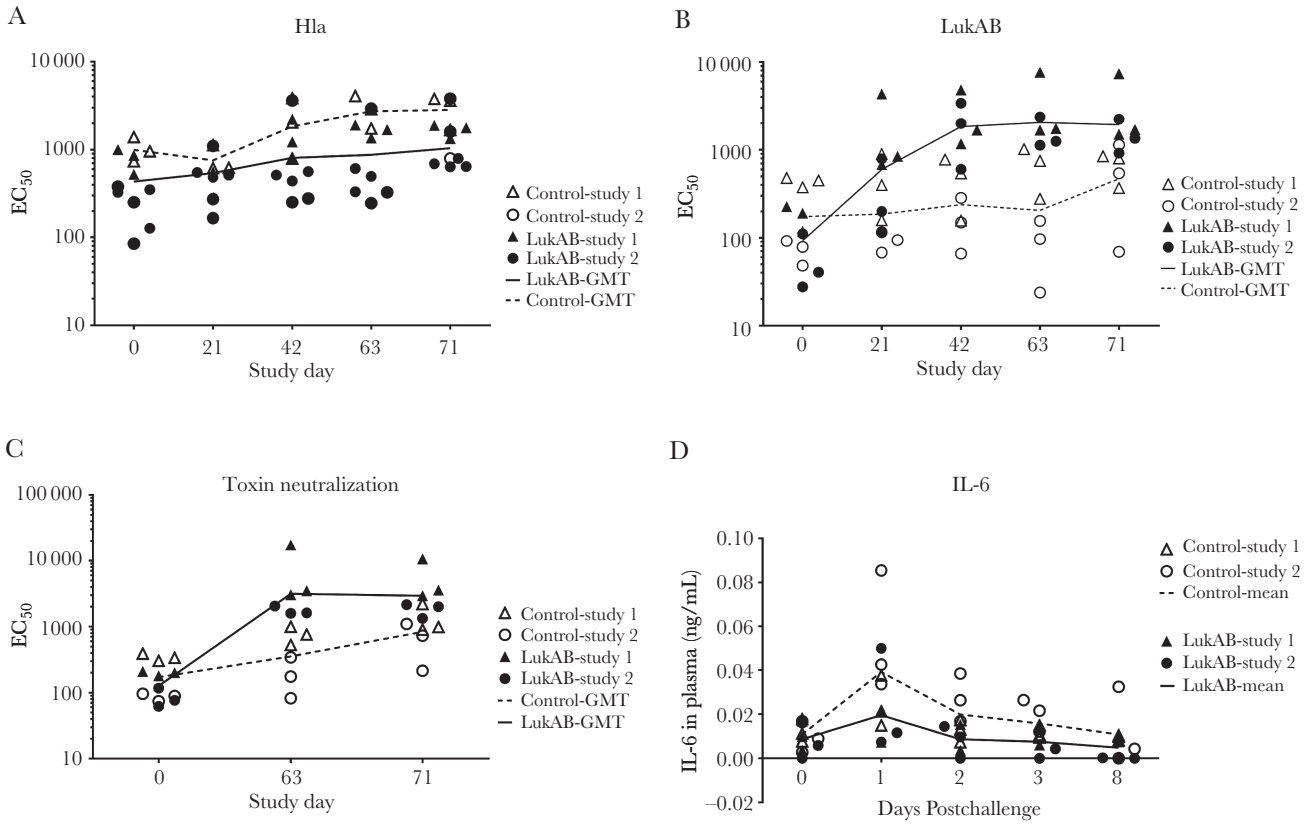

Figure 5. Humoral immunity generated after immunization with leukocidin AB (LukAB) toxoid, and systemic response to infection. Total immunoglobulin G was measured by enzyme-linked immunosorbent assay against Hla (*A*) and LukAB CC8 wild-type toxin (*B*). Ability of sera to neutralize cytotoxicity of LukAB was measured against THP-1 cells (*C*). Cytotoxicity was measured by lactate dehydrogenase release, and converted to a percentage relative to a toxin-only control. Concentrations of interleukin 6 were also measured in plasma prior to challenge and at postchallenge timepoints (*D*). In all cases, results as shown per animal per timepoint, with a line at the geometric mean (*A–C*) or mean (*D*) per group. Open circles/triangle and dashed lines indicate animals in the adjuvant-control group, while closed circles/triangles and complete lines indicate animals immunized with LukAB toxoid. The experiment was completed over 2 studies (3 animals per group per study = 6 animals per group total), with data points from each study shown as triangles (study 1) or circles (study 2) on the graphs. *A–C*, Study days are measured from the day of the first vaccination, ie, day 0, 21, and 42 = dose 1, 2, and 3, respectively; day 63 = challenge; day 71 = necropsy. *D*, Days after challenge, ie, 0 = challenge (study day 63); 8 = necropsy (study day 71).

infection sites as 2 independent measurable endpoints, with low-level infection in other organs.

Göttingen minipigs used in our studies had background antibodies to staphylococcal Hla, which increased with age, consistent with repeated exposure. This emulates similar changes in circulating anti-staphylococcal IgG observed in humans [46], but which is absent in mice. This may contribute to the artificially large protective effect of SA vaccines observed in mouse models that has not translated to protection in preexposed humans, or indeed in preexposed pigs.

To illustrate this point, we tested the protective efficacy of a vaccine containing capsular polysaccharides CP5 and CP8, both conjugated to CRM197, as an example of a vaccine that has shown protection in models in rodents but failed to show protection in humans, either alone [47], or in combination with 2 other antigens [48]. Both the CP5 and CP8 antigens were strongly immunogenic, but in our model did not show any protection compared to the negative control (as measured by bacterial burden at the surgical site and in the spleen). The ST398 challenge strain used is known to express capsule type CP5 in vitro, so it is expected that capsule is also expressed in vivo. As antibodies against the capsule are expected to function by promoting opsonophagocytosis, we compared the sera of vaccinated minipigs in an OPA assay against both the CP5-expressing ST398 challenge strain and a CP8-expressing clinical isolate. In both cases, minipig sera showed high levels of background OPA activity prior to immunization, and the presence of anti-CP5 or anti-CP8 antibodies in sera postvaccination did not markedly increase the OPA activity. This lack of increase in OPA over background could explain the lack of protection seen in this model with the CP5/8-CRM197 vaccine.

To further evaluate the model, we assessed a genetically detoxified LukAB vaccine that cannot be reliably tested in mouse models [13]. SA LukAB-mediated cytotoxicity on minipig granulocytes was similar to that observed with human PMNs, making minipigs a suitable model for evaluation of this toxin. Recently, Karauzum et al described a multicomponent toxoid vaccine containing a LukAB toxoid [12]. Although the authors

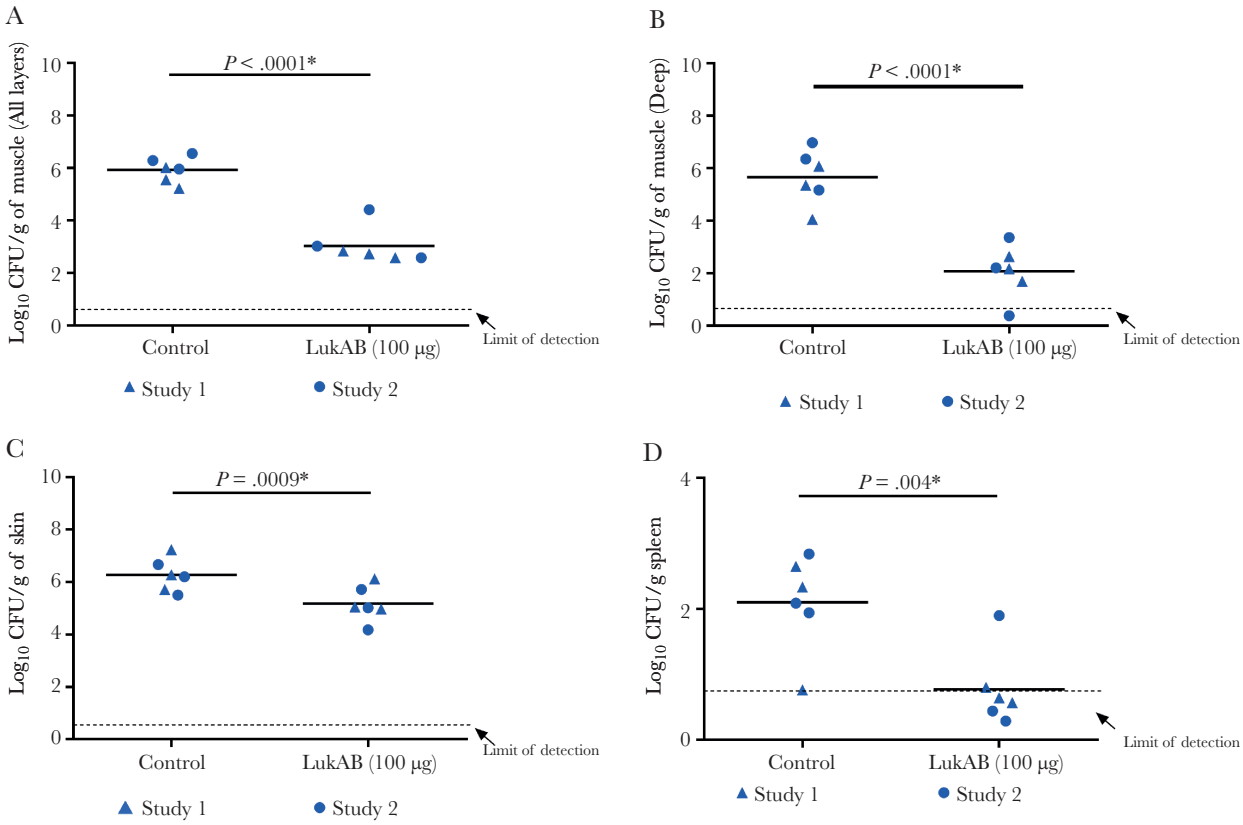

Figure 6. Effect of leukocidin AB toxoid in the muscle, skin, and spleen of the minipig. Bacterial burden after 8 days of infection (sequence type 398) in the combined surgical site muscle (*A*), deep surgical site muscle (*B*), skin (*C*), and spleen (*D*). *Significant difference between LukAB and control groups by Tobit regression model. Abbreviations: CFU, colony-forming units; LukAB, leukocidin AB.

show efficacy of their vaccine in murine and rabbit models of skin infection, the contribution of the anti-LukAB response to this effect was not evaluated. Since LukAB does not target murine cells and is only weakly active toward rabbit granulocytes [13, 15, 42], it is unlikely that the reported effect is due to LukAB targeting. Thus, it remains to be determined if targeting LukAB would prevent or ameliorate SA disease in vivo.

While the model described herein enabled us to study LukAB, many staphylococcal toxins/virulence factors show species specificity [13], and it is likely that there are SA virulence factors that do not "work" comparatively in pigs as in humans.

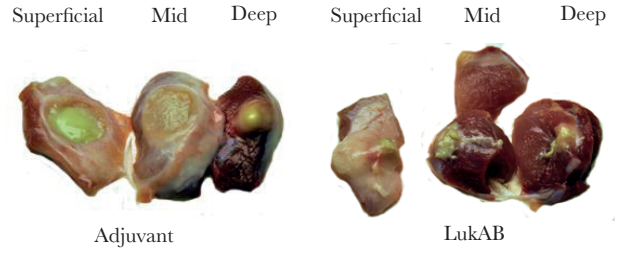

Figure 7. Effect of leukocidin AB (LukAB) toxoid in the muscle of the minipig. Representative photograph of purulent material at the surgical site 8 days after infection.

Published examples of these include staphylokinase [49] and chemotaxis inhibitory protein of SA (CHIPS) [50]. Therefore, although we expect the minipig model to be more suitable for a wider range of SA antigens than mice, there will still be antigens for which it is less effective. Furthermore, some humanized mouse models have been developed for studying particular antigens, such as humanized CD11b mice for studying LukAB [15], which can provide valuable answers to key questions on the antigens for which they were developed.

In contrast to CP5/8-CRM197, LukAB vaccination induced significant protection at the surgical site and in the spleen, and visibly reduced purulent material at the wound. The protective effect was reduced in the skin and superficial muscle, compared to deeper tissues, presumably due to the sutures acting as a nidus for the bacteria and allowing biofilm formation. The reduced bacterial burden in the spleens of the vaccinated group compared to the control group suggests that vaccination was furthermore able to reduce the dissemination of the bacteria from the wound. This reduced dissemination was associated with generally lower circulating levels of the inflammatory cytokine IL-6 in the days following infection.

Serum analysis showed that preexisting, neutralizing antibodies to LukAB were boosted by vaccination and by challenge

in the control group. Together, these results demonstrate that LukAB is produced by SA during both carriage and infection in pigs, further supporting the use of this model to evaluate LukAB vaccine candidates.

Our study shows that the minipig model warrants further investigation for other disease indications, and evaluation of additional virulence factors as vaccine antigens. This could include comparison of different LukAB toxoid candidates, such as those described by Kailasan et al [10]. However, there are limitations on the number of minipigs that can be used per study depending on the size of the vivarium, and the model might need to be supported by rodent and/or rabbit models to answer specific questions. Additionally, reagent availability for pigs is not as extensive as those for rodents, and the reagents for one breed of pig may not work for others.

In conclusion, Göttingen minipigs are an effective model for SA surgical wound infections and can be used with clinical isolates to evaluate the protective efficacy of SA vaccine candidates. We hope to use this model in future studies to evaluate different vaccine antigen combinations and formulations, as a reliable way to bridge preclinical results in mice to clinical results in humans.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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