Nontoxigenic protein A vaccine for methicillin-resistant *Staphylococcus aureus* infections in mice

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The current epidemic of hospital- and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections has caused significant human morbidity, but a protective vaccine is not yet available. Prior infection with *S. aureus* is not associated with protective immunity. This phenomenon involves staphylococcal protein A (SpA), an *S. aureus* surface molecule that binds to Fcγ of immunoglobulin (Ig) and to the Fab portion of Vγ3-type B cell receptors, thereby interfering with opsonophagocytic clearance of the pathogen and abating adaptive immune responses. We show that mutation of each of the five Ig-binding domains of SpA with amino acid substitutions abolished the ability of the resulting variant SpA\textsubscript{KKAA} to bind Fcγ or Fab Vγ3 and promote B cell apoptosis. Immunization of mice with SpA\textsubscript{KKAA} raised antibodies that blocked the virulence of staphylococci, promoted opsonophagocytic clearance, and protected mice against challenge with highly virulent MRSA strains. Furthermore, SpA\textsubscript{KKAA} immunization enabled MRSA-challenged mice to mount antibody responses to many different staphylococcal antigens.
RESULTS AND DISCUSSION

SpA is a virulence factor for lethal S. aureus infections

The contribution of the spa gene toward lethal S. aureus challenge has thus far not been appreciated. To address this, we generated the isogenic spa deletion variant S. aureus NewmanΔspa. After intraperitoneal challenge with 2 × 10^8 CFU of wild-type S. aureus Newman, 60% of animals succumbed to challenge. In contrast, animals infected with the isogenic mutant resulted in only 25% mortality (Fig. S1 A). In addition, the spa mutant displayed a consistent survival defect when examined in naive mouse blood (see Fig. 3 D). These results suggest that SpA is a crucial virulence factor for lethal infections of S. aureus in mice.

SpA-DKKAA cannot bind to immunoglobulin and trigger B cell apoptosis

Guided by amino acid homology, the triple α-helical bundle structure of Ig binding domains (Deisenhofer, 1981), and their atomic interactions with Fab VH3 (Graille et al., 2000) or Fcγ (Gouda et al., 1998), we selected glutamine 9 and 10, as well as aspartate 36 and 37, as critical for the association of SpA with immunoglobulin (Fig. 1, A and B; and Fig. S2, A and B). To test this, substitutions Gln 9Lys, Gln10Lys, Asp36Ala, and Asp37Ala were introduced into the D domain to generate SpA-DKKAA (Fig. 1 B). The ability of isolated SpA-D or SpA-DKKAA to bind human IgG or IgM was analyzed by affinity chromatography and ELISA (Fig. 1, C and D). Polyhistidine-tagged SpA-D, as well as full-length SpA, retained human IgG on Ni-NTA, whereas SpA-DKKAA or a negative control (sortase A; Mazmanian et al., 1999) did not (Fig. 1, C and D). A similar result was observed with vWF (Hartleib et al., 2000), which, along with TNFR1 (Gómez et al., 2004), can also bind SpA via glutamines 9 and 10 (Gómez et al., 2006; Fig. 1 D). Human Ig encompasses ~50% VH3-type IgG (Cook and Tomlinson, 1995). Human Fcy and F(ab)_2 fragments, as well as IgM, all

Silverman and Goodyear, 2006. In contrast, SpA binding to the Fcy of Ig interferes with opsonophagocytic clearance of staphylococci by polymorphonuclear leukocytes (Peterson et al., 1977). SpA is synthesized as a precursor with an N-terminal signal peptide and a C-terminal sorting signal for covalent anchoring to the cell wall (Schneewind et al., 1992). The N-terminal part of mature SpA is comprised of four or five 56–61-residue Ig binding domains (Sjödahl, 1977), which fold into triple helical bundles connected by short linkers (Deisenhofer, 1981). The C-terminal region X is comprised of Xr, a highly repetitive yet variable octapeptide, and Xc, a domain of unique sequence which abuts the cell wall anchor structure of SpA (Guss et al., 1984; Schneewind et al., 1995).

As a result of the attribute of simultaneously binding Fcy and Fab, SpA vaccines with neutralizing antibodies and protective immunity have hitherto not been reported (Greenberg et al., 1989). We wondered whether antibodies that neutralize the immunosuppressive properties of SpA could affect the outcome of S. aureus infections.

Figure 1. Generation of a nontoxicogenic SpA vaccine. (A) SpA of S. aureus Newman and USA300 LAC harbors an N-terminal signal peptide (white boxes), five Ig binding domains (E, D, A, B, and C), variable region X, and C-terminal sorting signal (black boxes). (B) Amino acid sequence of the five Ig binding domains, as well as nontoxicogenic SpA-DKKAA, with the positions of triple α-helical bundles (H1, H2, and H3), as well as glutamine (Q, red) 9 and 10 and aspartate (D, green) 36 and 37 as indicated. (C) Coomassie blue-stained SDS-PAGE of SpA, SpA-D, SpA-DKKAA, or sortase A purified on Ni-NTA sepharose in the presence or absence of human immunoglobulin (hIgG). (D) ELISA examining the association of immobilized SpA, SpA-D, or SpA-DKKAA with human IgG, as well as its Fc or F(ab)_2 fragments, vWF and IgM. Statistical significance of SpA-DKKAA binding to each ligand was compared against SpA-D, and SpA-D binding was compared against SpA (n = 3). *, P < 0.05; **, P < 0.01. (E) CD19+ B lymphocytes in splenic tissue of 6-wk-old BALB/c mice (n = 6) that had been mock immunized or treated with SpA-D or SpA-DKKAA were quantified by FACS. Data are the means and error bars represent ±SEM. Results in C–E are representative of three independent analyses.
bound to full-length SpA or SpA-D but not to SpA-DKKAA (Fig. 1 D). Injection of SpA-D into the peritoneal cavity of mice resulted in B cell expansion followed by apoptotic collapse of CD19+ (or CD45R+) lymphocytes in the spleen tissue of BALB/c or C57BL/6 mice (Goodyear and Silverman, 2003; Fig. 1 E). B cell superantigen activity was not detected after injection with SpA-DKKAA, and TUNEL-staining of splenic tissue failed to detect the increase in apoptotic cells that follows injection of SpA or SpA-D (Fig. 1 E; and Fig. S2, E–G).

**Antibodies against SpA-DKKAA protect against MSSA and MRSA infections**

Naïve BALB/c mice were injected with 50 µg each of purified SpA, SpA-D, or SpA-DKKAA emulsified in IFA. IgG responses to immunization were examined by ELISA with SpA-DGGSS and SpA-DKKAA. SpA-DGGSS harbors amino acid substitutions at the same positions as SpA-DKKAA; however, glutamines 9 and 10 were each replaced with glycine and aspartic acids 36 and 37 with serine. Similar to SpA-DKKAA, SpA-DGGSS does not interact with human IgG (unpublished data). It is of note that similar antibody titers were measured with SpA-DGGSS and SpA-DKKAA antigen, indicating that the four amino acid substitutions do not diminish the reactivity of antibodies raised with heterologous antigens (IgG titers against SpA-DKKAA vs. SpA-DGGSS, P = 0.8315). After immunization of mice with either SpA-D or SpA-DKKAA, we observed a 10-fold higher titer of SpA-specific antibodies for the nontoxicogenic variant as compared with the B cell superantigen (P < 0.0001; Table I). Antibody titers raised by immunization with full-length SpA were higher than those elicited by SpA-D (P = 0.0022), which is likely a result of the larger size and reiterated domain structure of this antigen (Table I). Nevertheless, even SpA elicited lower antibody titers than SpA-DKKAA (P = 0.0003), which encompasses only 50 aa of the mature 520-residue protein. Immunized mice were challenged by intravenous inoculation with *S. aureus* Newman, and the ability of staphylococci to seed abscesses in renal tissues was examined by necropsy 4 d after challenge (Cheng et al., 2009).

In homogenized renal tissue of mock (PBS/adjuvant) immunized mice, a mean staphylococcal load of 6.46 log10 CFU g⁻¹ was enumerated (Table I). Immunization of mice with SpA or SpA-D led to a reduction in staphylococcal load; however, SpA-DKKAA–vaccinated animals displayed an even greater 3.07 log10 CFU g⁻¹ reduction of *S. aureus* Newman in renal tissues (P < 0.0001; Table I). Abscess formation in kidneys was analyzed by histopathology (Fig. S3 A–H). Mock immunized animals harbored a mean of 3.7 (±1.2) abscesses per kidney (Table I). Vaccination with SpA-DKKAA reduced the mean number of abscesses to 0.5 (±0.4; P = 0.0204), whereas immunization with SpA or SpA-D did not cause a significant reduction in the number of abscess lesions (Table I). Lesions from SpA-DKKAA–vaccinated animals were smaller in size, with fewer infiltrating PMNs, and characteristically lacked staphylococcal abscess communities (Cheng et al., 2009; Fig. S3, A–H). Abscesses in animals that had been immunized with SpA or SpA-D displayed the same overall structure of lesions in mock immunized animals (Fig. S3, A–H).

We wondered whether SpA-DKKAA immunization could protect mice against MRSA strains and selected the USA300 strain for further studies. To evaluate whether SpA-DKKAA bound to full-length SpA or SpA-D but not to SpA-DKKAA (Fig. 1 D). Injection of SpA-D into the peritoneal cavity of mice resulted in B cell expansion followed by apoptotic collapse of CD19⁺ (or CD45R⁺) lymphocytes in the spleen tissue of BALB/c or C57BL/6 mice (Goodyear and Silverman, 2003; Fig. 1 E). B cell superantigen activity was not detected after injection with SpA-DKKAA, and TUNEL-staining of splenic tissue failed to detect the increase in apoptotic cells that follows injection of SpA or SpA-D (Fig. 1 E; and Fig. S2, E–G).

### Table 1. Active immunization of mice with SpA vaccines

<table>
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<tr>
<th>Antigen</th>
<th>Staphylococcal load and abscess formation in renal tissue</th>
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<td></td>
<td>Log₁₀ CFU g⁻¹ᵃ</td>
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<td>S. aureus Newman challenge</td>
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<tr>
<td>Mock</td>
<td>6.46 ± 0.25</td>
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<tr>
<td>SpA</td>
<td>3.95 ± 0.56</td>
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<tr>
<td>SpA-D</td>
<td>4.43 ± 0.41</td>
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<td>SpA D_KKAA</td>
<td>3.39 ± 0.50</td>
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<td>S. aureus USA300 (LAC) challenge</td>
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<tr>
<td>Mock</td>
<td>7.20 ± 0.24</td>
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<tr>
<td>SpA</td>
<td>6.81 ± 0.26</td>
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<tr>
<td>SpA-D</td>
<td>6.34 ± 0.52</td>
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<tr>
<td>SpA-D_KKAA</td>
<td>6.00 ± 0.42</td>
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<td>SpA_KKAA</td>
<td>3.66 ± 0.76</td>
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ᵃMeans ± SEM of staphylococcal load calculated as log₁₀ CFU g⁻¹ in homogenized renal tissues 4 d after infection in cohorts of 15–20 BALB/c mice per immunization.
ᵇStatistical significance was calculated with the unpaired two-tailed Students’ t-test and P-values were recorded.
ᶜReduction in bacterial load calculated as log₁₀ CFU g⁻¹.
ᵈMeans ± SEM of five randomly chosen serum IgG titers were measured before staphylococcal infection by ELISA using SpA-D_KKAA or SpA-DGGSS as antigens.
ᵉHistopathology of hematoxylin-eosin–stained thin-sectioned kidneys from 10 animals. The mean number of abscesses per kidney was recorded and averaged again for the final mean ± SEM.
SpA-DKKAA antibodies neutralize immunoglobulin binding activities of SpA

Rabbits were immunized with SpA-DKKAA, and specific antibodies were purified on SpA-DKKAA affinity column and analyzed by SDS-PAGE (Fig. 2 A, lane 1). SpA-DKKAA antibodies were treated with pepsin to separate Fc and F(ab)2 (lane 2). The resulting F(ab)2 fragments were repurified by affinity chromatography on SpA-DKKAA (lane 3).

Binding of human IgG or vWF to SpA or SpA-D was perturbed by SpA-DKKAA-specific F(ab)2, indicating that SpA-DKKAA-derived antibodies can block the Fc- and vWF-binding properties of SpA (Fig. 2, B and C).

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Two independent analyses.

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A, B, and C; Sjödahl, 1977). Polyhistidine-tagged SpAKKAA was purified by affinity chromatography and analyzed by Coomassie blue–stained SDS-PAGE (Fig. 3 A). Unlike full-length SpA, SpAKKAA did not bind human IgG, Fc and F(ab)2, IgM, or vWF (Fig. 3 B). SpAKKAA failed to display B cell superantigen activity, as injection of the variant into BALB/c or C57BL/6 mice did not cause a depletion of both CD19+ and CD45R+ B cells in splenic tissue (Fig. 3 C; Fig. S2, C and D; and not depicted). SpAKKAA immunization generated higher specific antibody titers than SpA-DKKAA and provided mice with elevated protection against S. aureus USA300 (Table I) or Mu50 challenge (Fig. S3 U; Mu50 is a Japanese MRSA isolate). 4 d after challenge, SpAKKAA-vaccinated animals harbored 3.54 log10 CFU g⁻¹ fewer staphylococci in renal tissues (P = 0.0001) and also caused a greater reduction in the number of abscess lesions (P = 0.0109; Table I; and Fig. S3, F–R). Furthermore, SpAKKAA immunization reduced the mortality of mice that received a lethal S. aureus challenge dose (Fig. S1 B).

Staphylococci persist in mouse organ tissues (Cheng et al., 2009). Over time, abscesses harboring communities of the infectious agent increase in size and eventually rupture, thereby releasing staphylococci into circulation. This initiates the formation of new abscesses and precipitates lethal outcomes 30–60 d after challenge (Cheng et al., 2009). We asked whether SpA-DKKAA or SpAKKAA immunization prevents staphylococcal replication over a longer period of time. 15 d after challenge with S. aureus USA300, immunization with SpA did not generate significant protection of animals compared with the mock control (P = 0.5817; Table S1). In contrast, immunization with SpA-DKKAA caused a 1.56 log10 CFU g⁻¹ reduction (P = 0.0183), whereas SpAKKAA vaccination reduced the load by 2.7 log10 CFU g⁻¹ (P = 0.0059). These data suggest that antibodies against SpA, generated via active immunization using nontoxigenic SpAKKAA, can interfere with bacterial persistence in host tissues.

**SpA-specific antibodies generate protective immunity**

SpAKKAA was used to immunize rabbits. Rabbit antibodies specific for SpA-DKKAA or SpAKKAA were affinity purified on matrices with immobilized cognate antigen and injected at a concentration of 5 mg × kg⁻¹ body weight into the peritoneal cavity of BALB/c mice (Table II). 24 h later, antibody titers specific for SpA-DKKAA/SpAKKAA were determined in serum and animals challenged by intravenous inoculation with S. aureus Newman. Passive transfer reduced the staphylococcal load in kidney tissues for SpA-DKKAA (P = 0.0016) or SpAKKAA (P = 0.0005)–specific antibodies. On histopathology examination, both antibodies reduced the abundance of abscess lesions in kidneys of mice challenged with S. aureus Newman (Table II). Compared with control cohorts treated with non-specific antibody (α-V10), animals that had been injected with SpA-specific antibodies were protected against the B cell superantigen activity of SpA (Fig. S2, C and D). In addition, SpA-specific antibodies (2 µg × ml⁻¹) induced opsonophagocytic clearance of S. aureus Newman inoculated into naive mouse blood (Fig. 3 D) and reduced the mortality associated with lethal staphylococcal challenge (Fig. S1 C). Together these data reveal that disease protection after immunization with SpA-DKKAA or SpAKKAA is conferred by antibodies that bind SpA and neutralize its ability to bind Ig.

**SpAKKAA immunization promotes host antibody response to staphylococcal infection**

After infection with virulent S. aureus Newman and clearance of the pathogen with antibiotic treatment, mice do not develop protective immunity against subsequent infection with the same strain (Fig. S3 S). The mean abundance of SpA-DKKAA–specific IgG in these animals was determined by dot blot as 0.20 µg ml⁻¹ (±0.04) and 0.14 µg ml⁻¹ (±0.01) for infections caused by S. aureus strains Newman and USA300 LAC, respectively (Fig. 4 A). A concentration of 4.05 µg ml⁻¹ (±0.88) for SpA-specific IgG was estimated to confer disease protection in SpAKKAA- or SpA-DKKAA–immunized mice (P ≤ 0.05 log10 reduction in staphylococcal CFU g⁻¹ renal tissue; unpublished data). The mean serum concentration of SpA-specific IgG in adult healthy human volunteers (n = 16) was 0.21 µg ml⁻¹ (±0.02). Such antibody concentration may not be sufficient to generate protection against staphylococcal infections.
Infection. By comparison, the mean serum concentration of IgG specific for diphtheria toxin in human volunteers, 0.68 µg ml⁻¹ (± 0.20), is thought to be within range for protective immunity against diphtheria (Lagergård et al., 1992).

These results are in agreement with a model of immune evasion during S. aureus infection. Cell wall–anchored or secreted SpA (e.g., 20% of peptidoglycan and attached surface protein) is released during bacterial division; Ton-That et al., 1999) activate B cells via IgM receptor cross-linking. Without stimuli from specific antigens, activated B cells undergo apoptosis, thereby hindering the production of antibody against staphylococcal antigens. If so, neutralizing antibodies directed against SpA may enable humoral immune responses against many different staphylococcal antigens. This was tested by immunizing BALB/c mice with SpAKKAA or an adjuvant (aluminum hydroxide) control, followed by intravenous challenge with a sublethal dose of MRSA strain USA300. Serum samples were withdrawn 30 d after MRSA challenge and then analyzed by immunoblotting with 27 staphylococcal antigens immobilized on a membrane filter (Fig. 4 B). Naive mice, which had not been infected with the MRSA strain USA300 LAC, did not harbor antibodies against staphylococcal antigens (unpublished data). Mock immunized mice (adjuvant only) that had been subjected to USA300 infection developed higher antibodies against the Eap protein as well as low-titer antibodies against Hla, IsdA, IsdB, LukD, LukE, and LukF (Fig. 4 B). In response to USA300 challenge, animals that had been immunized with SpAKKAA (IgG titer 2,907 ±357; P < 0.001, SpAKKAA vs. mock) mounted humoral immune responses against every antigen examined (Fig. 4 B). With the exception of Eap, IsdA, and IsdB antibodies, the serum of SpAKKAA-immunized animals harbored higher antibody titers against staphylococcal antigens as compared with mice that had been naive at the time of challenge (Fig. 4 B).

In summary, S. aureus isolates express SpA, an essential virulence factor whose B cell superantigen activity and evasive attributes toward opsonophagocytic clearance are required for staphylococcal abscess formation and the establishment of lethal disease (Cheng et al., 2009). SpA can be thought of as a toxin that is essential for pathogenesis and whose molecular attributes must be neutralized to achieve protective immunity.

By generating nontoxigenic variants unable to bind Igs via Fcγ or VH3-Fab domains, we identified SpA-neutralizing immune responses as a correlate for protective immunity against S. aureus infection. In contrast to many methicillin-sensitive strains, the CA-MRSA isolate USA300 LAC is significantly more virulent (Cheng et al., 2009). For example, immunization of experimental animals

![Figure 4.](https://example.com/figure4.png)
with the surface protein IsdB (Kuklin et al., 2006) raises antibodies that confer protection against S. aureus Newman (Kuklin et al., 2006) but not against USA300 challenge (Fig. S3 T). In contrast, neutralizing SpA antibody responses generate protection against strains of the current MRSA epidemic. These antibodies exert at least two functions. As shown in Fig. 3, the SpA antibodies enable phagocytic killing of staphylococci in blood. Moreover, by neutralizing B cell superantigen activity, SpA antibodies enable the development of humoral immune responses to many different antigens that, assuming synergism, may together contribute toward the establishment of immunity. In agreement with this, the SpA<sub>KKAA</sub> vaccine elicited greater protection against abscess formation, which monitors infected animals over a prolonged period of time (Fig. 4), as compared with the lethal challenge, when most animals die within 1–2 d (Fig. S1).

**MATERIALS AND METHODS**

**Antibody isolation.** 5 mg of protein was covalently linked to HiTrap NHS-activated HP and loaded with rabbit serum. Antibodies were eluted with 1 M glycine, pH 2.5, and neutralized with 1 M Tris-HCl, pH 8.5, and dialyzed against PBS. Affinity-purified antibodies were mixed with 1 M glycine, pH 2.5, and 0.5 M NaCl, neutralized with 1 M Tris-HCl, pH 8.5. F(ab)<sub>2</sub> fragments were affinity purified, dialyzed against PBS at 4°C, separated by 15% SDS-PAGE, and visualized with Coomassie Blue.

**Active and passive immunization.** The coding sequence for SpA was PCR amplified with two primers, 5′-GCGTCACATGGCCACAGTG-GAGGCTCAAC-3′ and 5′-AGTTGAATGAAG-3′ with two primers: 5′-GAAGCTCAAC-3′ and 5′-GCACTTGC-3′, using S. aureus Newman DNA. SpA-D was PCR amplified with two primers: 5′-AAAGATGCTCAACAAAGCAATCAAAAGC-3′ and 5′-AAGGATCAGTATGTTTAATCGTTTTGTCG-3′. The sequence for SpA<sub>D</sub> was mutated with two sets of primers: 5′-CATATGTCACAACAAAGATAAATCAAGGC-3′ and 5′-GATTTCTATAGAAAGGGCTCCTATTCTGAACTC-3′ and 5′-GATTTCTATAGAAAGGGCTCCTATTCTGAACTC-3′. The sequence for SpA<sub>Δ</sub> was synthesized by Integrated DNA Technologies, Inc. PCR products were cloned into pET-15b generating N-terminal His<sub>6</sub>-tagged recombinant protein. BALB/c mice were immunized by intramuscular injection and boosted with the same antigen after 11 d. On day 20, mice were bled to obtain serum for specific antibody titters. Affinity-purified antibodies were injected into the peritoneal cavity of BALB/c mice at 4–24 h, and blood was collected for specific antibody titters before challenge.

**Mouse infection.** Staphylococci were used to infect anesthetized mice by retroorbital injection (1 × 10<sup>6</sup> CFU S. aureus Newman, 5 × 10<sup>6</sup> CFU S. aureus USA300, or 3 × 10<sup>7</sup> CFU S. aureus Mu50). On day 4, 15, or 30, mice were killed, kidneys removed, and homogenized tissue spread on agar for colony formation. Animal experiments were performed in agreement with the institutional guidelines according to experimental protocol review and approval by the Institutional Biosafety Committee and the Institutional Animal Care and Use Committee at the University of Chicago.

**Bacterial survival in blood.** As previously described (Thammanongs and others, 2009), whole blood was collected from BALB/c mice with 5 µg ml<sup>-1</sup> of lepirudin anticoagulant. 50 µl of 5 × 10<sup>8</sup> CFU ml<sup>-1</sup> S. aureus Newman were mixed with 950 µl of mouse blood. Samples were incubated at 37°C with slow rotation for 30 min and then incubated on ice with 1% saponin/PBS. Dilutions of staphylococci were plated on agar for colony formation.

**SpA ligands.** 1 µg ml<sup>-2</sup> of purified SpA and its variants were coated onto ELISA plates in 0.1 M carbonate buffer, pH 9.5. Plates were incubated with peroxidase-conjugated human IgG, Fe, or F(ab)<sub>2</sub> fragments, IgM (The Jackson Laboratory), and vWF (Thermo Fisher Scientific) and developed using OPD/EIA reagent. For inhibition, plates were incubated with either naïve rabbit F(ab)<sub>2</sub> fragments (The Jackson Laboratory) or 10 µg ml<sup>-1</sup> of affinity-purified F(ab)<sub>2</sub> fragments before ligand binding.

**B cell apoptosis.** 150 µg of purified protein was injected into the peritoneum of 6-wk-old BALB/c. 4 h after injection, animals were killed and spleens removed and homogenized. Red blood cells were lysed in ACK buffer. White blood cells were stained with R-PE-conjugated anti-CD19 (eBioscience). Cells were washed and fixed with formalin and analyzed by FACScanto (BD).

**Antibody quantification.** Nitrocellulose membrane was blotted with human/mouse IgG (The Jackson Laboratory), SpA<sub>KKAA</sub> and CRM<sub>197</sub>, blocked, and incubated with either human or mouse sera. IRDye 700DX–conjugated anti-human/mouse IgG (Rockland Immunochemicals, Inc.) was used to quantify signal intensities from healthy human volunteers or mice using the Odyssey infrared imaging system (LI-COR, Biosciences). Experiments with blood from human volunteers involved protocols that were reviewed, approved, and performed under regulatory supervision of The University of Chicago’s Institutional Review Board. For the staphylococcal antigen matrix, nitrocellulose membrane was blotted with 2 µg of a collection of Ni-NTA affinity-purified recombinant SpA ligands. Signal intensities in mouse sera were quantified and normalized using anti-His antibody with the Odyssey.

**Statistical Analysis.** Unpaired two-tailed Student’s t tests were performed to analyze the statistical significance of renal abscess, ELISA, and B cell superantigen data.

**Online supplemental material.** Fig. S1 shows that SpA is a virulence factor for lethal infection after intraperitoneal injection of S. aureus Newman into BALB/c mouse and that active or passive immunization with antibodies raised against SpA<sub>KKAA</sub> can protect against this disease. Fig. S2 shows that SpA<sub>KKAA</sub>, unlike wild-type SpA, does not induce B cell apoptosis in mice and that antibodies raised against SpA<sub>KKAA</sub> neutralize the B cell superantigen attributes of SpA. Fig. S3 shows that immunization of mice with SpA<sub>KKAA</sub> prevents multiple S. aureus strains from inducing renal abscess formation in mice and that prior infection with S. aureus does not elicit immunity to subsequent infection with the same strain. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20092514/DC1.

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