Demonstration of the preclinical correlate of protection for Staphylococcus aureus clumping factor A in a murine model of infection

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A B S T R A C T

The Staphylococcus aureus virulence factor clumping factor A (CIFA) is a component of an investigational S. aureus prophylactic vaccine. CIFA enables S. aureus to bind to fibrinogen and platelets during the initial stages of invasive disease. Here we demonstrate that ectopic expression of CIFA is sufficient to render nonpathogenic Lactococcus lactis lethal in a murine model of systemic infection. In contrast, L. lactis expressing CIFA\textsuperscript{Y338A}, which cannot bind fibrinogen, did not cause death in the mice. Pathogenicity was also prevented by immunization with CIFA. This model was then used to define a preclinical correlate of protection by measuring functional antibody in a S. aureus fibrinogen binding inhibition assay (FBI) and correlating that titer with protective outcomes. Although many humans have pre-existing antibodies that bind to CIFA, only sera with a threshold functional titer in the FBI were protective in this preclinical model. This confirms that fibrinogen binding is critical for CIFA-mediated pathogenesis and demonstrates that functional antibodies against CIFA are sufficient to protect against CIFA-mediated pathogenesis in vivo, enabling the definition of a preclinical correlate of protection for CIFA-containing vaccines based on FBI titer.

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1. Introduction

\textit{Staphylococcus aureus} is a medically important Gram positive coccus that is carried asymptomatically in the nares of 20–50% of individuals [1]. \textit{S. aureus} causes a wide spectrum of disease ranging from relatively mild skin infections, such as impetigo, to life-threatening bloodstream infections, and is recognized as a leading cause of serious disease in both community and healthcare-associated settings. In surgical patients in particular, infections are associated with high mortality rates. Survivors of \textit{S. aureus} surgical infections require hospital stays three times longer than uninfectected patients, significantly increasing healthcare costs [2]. The burden of \textit{S. aureus} disease is exacerbated by antibiotic resistance in \textit{S. aureus} isolates, highlighting the need for an effective prophylactic vaccine.

To establish infection, \textit{S. aureus} must adhere to host cells, extracellular matrix, or an implanted medical device. \textit{S. aureus} clumping factor A (CIFA)\textsuperscript{1} is a member of the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family and plays an important role in establishing wound and foreign body infections. Originally identified by Foster and colleagues [3], CIFA derives its name from its ability to induce clumping of \textit{S. aureus} in the presence of fibrinogen. Although \textit{S. aureus} possesses many adhesins, CIFA is the major fibrinogen-binding protein in \textit{S. aureus} [4,5], binding to the C-terminal end of the fibrinogen \textit{γ}-chain. CIFA is essential during the early stages of infection and its importance as a \textit{S. aureus} virulence factor has been demonstrated in several small animal models of infection, including endocarditis, arthritis, and sepsis [6–8]. Humans are routinely exposed to \textit{S. aureus}, and develop binding antibodies to CIFA and other surface exposed proteins, however they generally do not develop functional antibodies.

\textsuperscript{1} Abbreviations: CIFA: Clumping factor A, MSCRAMM: microbial surface component recognizing adhesive matrix molecules, FBI: fibrinogen binding inhibition assay, rCIFA: recombinant CIFA, rmCIFA: recombinant CIFA containing a Y338A mutation.

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that can inhibit the binding of the whole bacteria to fibrinogen [9]. This may be a factor in the failure of passive immunotherapy clinical trials that utilized CIWA enriched antibody preparations from unvaccinated donors [10]. In contrast, vaccination of small animals and humans with a multi-component vaccine that includes recombinant CIWA protein was able to induce antibodies that prevented the binding of S. aureus to fibrinogen using the fibrinogen binding inhibition (FBI) assay [9].

In this study, we employed the *Lactococcus lactis*:ClfA ectopic expression model [11,12] which showed that CIWA is a critical virulence factor capable of conferring a lethal phenotype upon an avirulent microorganism in a murine systemic infection model. Pathogenicity specifically required CIWA-mediated binding to fibrinogen. CIWA antibodies that prevent the binding of *S. aureus* to fibrinogen *in vitro* abrogated *L. lactis*:CIWA pathogenicity *in vivo*. Immunization with CIWA induced a protective response in mice challenged with *L. lactis*:CIWA. By titrating CIWA immune sera with known FBI titer, a threshold protective titer was defined. This FBI threshold protective titer can be used as a preclinical correlate of protection for CIWA-containing vaccines.

2. Materials and methods

2.1. Cloning and expression of recombinant ClfA proteins

Enzymes for DNA manipulation were obtained from New England Biolabs and Takara Bio and primers from Integrated DNA Technologies. DNA manipulations were performed using standard procedures. Synthetic codon optimized DNA sequence (Blue Heron) corresponding to CIWA amino acids 40–559 (domains N1N2N3, rClfA) from *S. aureus* PFE30043 was subcloned into a pET30a vector. A CIWA variant lacking fibrinogen-binding activity (rClfA) was constructed by DNA synthesis and replacement. Following confirmation of DNA sequence, the wild type rClfA N1N2N3 fragment and the corresponding rClfA fibrinogen binding mutant were expressed as recombinant proteins in *Escherichia coli*. Proteins were purified using a combination of hydrophobic and anion-exchange chromatography [9], quantitated with a bichinchoninic acid assay (Pierce Biochem), purity assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and endotoxin levels analyzed using the Limulus amebocyte lysate assay (Charles River).

2.2. L. lactis strains

*L. lactis* cultures were grown in M17 media (Difco). The pKS80 plasmid coding for wild type *S. aureus* CIWA (pKS80/cfA) was kindly provided by T. Foster and used to generate the *L. lactis*:ClfA strain. Plasmid DNA was isolated from *L. lactis* via Qiagen Plasmid maxikit (Qiagen) per manufacturer’s instructions, except that cells were grown to late exponential phase, harvested and resuspended in THMS (30 mM Tris–HCl pH 8, 3 mM MgCl₂ in 25% sucrose) supplemented with 2 mg/ml lysozyme. A CIWA variant lacking fibrinogen-binding activity was constructed by introducing the Y338A amino acid substitution by overlap primer polymerase chain reaction (PCR) using pKS80/cfA as template. A ~600 bp BpmI/BclI restriction enzyme fragment spanning the Y338A amino acid substitution was then subcloned into pKS80/cfA. The amino acid substitution was sequence-verified, and used to generate the *L. lactis*:ClfAm strain.

2.3. Flow cytometry

Bacteria were heat-killed by treatment for one hour at 60°C and then blocked with 10% porcine serum/PBS to prevent nonspecific antibody binding. Mouse CIWA monoclonal antibody or isotype control was added (20 µg/ml) and incubated for 30 min on ice. Cells were then sequentially incubated with biotinylated anti-mouse IgG (Jackson Immunoresearch), streptavidin–PE (BD Biosciences) and then with a series of washes using 10% porcine serum/PBS. Pellets were resuspended in 1% paraformaldehyde. A total of 20,000 events per well were acquired on a BD LSR II flow cytometer and analyzed using FlowJo v10 software (Tree Star). The mean fluorescence intensity (MFI) of the PE channel was determined for each sample after gating on bacterial cells in the logarithmic FSC versus SSC dot plot. A sample was considered positive if the MFI value was at least three times the control mouse IgG MFI and greater than 100.

2.4. Fibrinogen binding assay

Human fibrinogen (Fg) (Calbiochem) was immobilized onto microtiter plates by incubating 100 µL/well of 3 µg/ml fibrinogen solution in TBS, pH 7.4 buffer overnight at 4°C, and either 5 µM rClfA or rmClfA were added into the wells. Unbound rClfA or rmClfA was removed by washing microtiter plates with TBS, pH 7.4 buffer containing 0.05% Tween 20 (TBS-Tween), and bound rClfA or rmClfA was detected with polyclonal mouse anti-rClfA antibody, followed by a secondary detection antibody linked to alkaline phosphatase (SouthernBiotec). Signal was developed by adding pNPP substrate (KPL), and absorbance at 405 nm was read on a Spectramax Plus spectrophotometer (molecular devices).

2.5. Fibrinogen binding inhibition assay

Fg binding inhibition assays were performed as previously described [9]. Briefly, microtiter plate wells were coated with 1.7 µg/mL human Fg (Calbiochem) and then incubated with blocking solution (MBA, GE Healthcare) to reduce nonspecific binding. *L. lactis* isolates (10⁶–10⁷ CFU) were either added directly to the Fg-coated plate or preincubated with antisera and subsequently transferred to the Fg coated plate. After 30 min at 37°C, wells were washed to remove non-adherent cells, and adherent cells were quantified using the luciferase-based BacTiter-Glo® lysis reagent (Promega) and read on an Envision 2100 Multilabel Reader (Perkin Elmer L100) or Spectramax luminometer Model L (Molecular Devices).

2.6. Murine challenge experiments

All animal work was performed in strict accordance with approved Institutional Animal Care and Use Committee (IACUC) protocols at an American Association of Laboratory Animal Science (AALAS) accredited facility. Female 6–8 week old BALB/c mice (Charles River Laboratories) were used for active immunization experiments, and 10–12 week old mice were used for preincubation, passive immunization, and challenge studies. Number of animals per group is indicated in the legend of each figure. For active immunization experiments, mice were immunized subcutaneously at weeks 0, 3, and 6 with rmClfA + Al(OH)₃, or Al(OH)₃ alone. Mice were bled on days 0 and 56, and challenged with *L. lactis*:CIWA on day 60. For passive immunization experiments, the animals were administered 1.5 mg of the functional anti-CIFA mAb 12–9 or PBS control via intraperitoneal injection (IP) 16 h prior to bacterial challenge. For preincubation experiments, ~1 × 10⁶ CFU of *L. lactis*:CIFA bacteria were preincubated for two hours at 4°C with varying amounts of the 12–9 mAb (10, 15, 25, 50, or 100 µg), 100 µg of the CIFA mAb 15SEC6 [13], 100 µg of control mAb, varying amounts of the mouse polyclonal CIFA antibody, or PBS. Bacteria were pelleted by centrifugation (4000 × g, 4°C, 15 min), the supernatant containing unbound mAb was decanted, and the bacterial pellet was resuspended in PBS for injection. Mice were challenged with ~1 × 10⁶ CFU/mouse of *L. lactis*:vector, *L. lactis*:CIFA, or *L. lactis*:CIWA via tail vein injection.
and survival was monitored for 7 days post challenge. Data were analyzed using GraphPad Prism software (GraphPad Software, Inc.).

2.7. Murine gross necropsy and tissue histopathology

Three *L. lactis*:ClfA and two *L. lactis*:vector control infected mice were examined for gross findings at necropsy. At necropsy, liver, spleen, lung, gut, kidney, and pancreas were collected and fixed in 10% Neutral Buffered Formalin (NBF). After the tissues were fixed for 24 h in 10% NBF, they were cassetted individually to maintain identity and processed whole on a Sakura VIP 5 series by dehydrating through a series of graded ethanol solutions, cleared with xylene and impregnated with paraffin. The tissues were embedded in block and sectioned in 4 μm thickness. Sectioned tissues were heated in a 60 degree oven for a minimum of 1 h, stained via automated linear stabilizer with hematoxylin–eosin (HE) and coverslipped. Slides were examined by a trained pathologist and images captured on an EVOS® FL Auto Cell Imaging System (Life Technologies) using EVOS® FL Auto Software Revision 1.6 (Life Technologies).

3. Results

3.1. ClfA-dependent binding of *L. lactis* to human fibrinogen

Adhesion to host substrate(s) is an early step in the establishment of an invasive bacterial infection, and *S. aureus* has multiple redundant mechanisms that enable binding to host factors in vivo. To experimentally probe the role of ClfA in adhesion in isolation, we expressed *S. aureus* ClfA in the nonpathogenic bacterium *L. lactis*. Unlike *L. lactis* bearing only the expression vector (*L. lactis*:vector), expression of ClfA is detected on the surface of *L. lactis*:ClfA by flow cytometry (Fig. 1A). ClfA expression on two *S. aureus* clinical isolates is shown for comparison. Both recombinant ClfA (rClfA) in isolation (Fig. 1B) and rClfA expressed on the surface of *L. lactis* (*L. lactis*:ClfA, Fig. 1C) bound to immobilized human fibrinogen.

While ClfA is the primary fibrinogen-binding adhesion factor of *S. aureus*, ClfA has also been described to bind Factor I and facilitate integrin binding, which may contribute to ClfA-mediated virulence [4,14,15]. Loughman et al. had previously shown that the introduction of two amino acid substitutions into the ClfA A domain abrogates fibrinogen binding [16]. To ask whether fibrinogen binding is fully or only partially responsible for ClfA-mediated virulence, a single point mutation was introduced into the fibrinogen-binding A domain of clfA, to generate a Y338A substitution in the gene product. The Y338A substitution did not impact ClfA surface expression as detected by flow cytometry (Fig. 1A), but did abrogate binding to immobilized fibrinogen, whether coupled to Sepharose on an affinity chromatography column (data not shown), or bound to plastic on a microtiter plate (Fig. 1B). Likewise, *L. lactis* strains bearing the nonrecombinant expression vector (*L. lactis*:vector) or expressing the Y338A substitution mutant (*L. lactis*:ClfAm) were unable to bind to fibrinogen in vitro (Fig. 1C).

3.2. ClfA-dependent virulence in mice challenged with *L. lactis*:ClfA

Que et al. [11] have reported that heterologous expression of *S. aureus* ClfA enhances *L. lactis* pathogenicity in a rat endocarditis model. To better understand ClfA-mediated virulence in vivo, mice were challenged systemically with *L. lactis*:ClfA or
the *L. lactis*:vector control, and survival was monitored. While there was no detectable virulence in mice challenged with the *L. lactis*:vector organisms, the *L. lactis*:CIFA organisms were highly virulent, with most animals succumbing to the infection within 24 h (Fig. 2). Necropsies with tissue histopathology were conducted after challenge which demonstrated systemic bacteremia in the mice challenged with the CIFA expressing *L. lactis* strain, but not in mice challenged with *L. lactis* alone (Supplemental Fig. 1).

Virulence of the *L. lactis*:CIFAm strain was then compared to the wild type *L. lactis* strain in the mouse systemic infection model. Female BALB/c mice were challenged IV with −1 × 10^9 CFU of *L. lactis*:vector, *L. lactis*:CIFA, or *L. lactis*:CIFAm. Strikingly, the single Y338A substitution in CIFA rendered the *L. lactis*:CIFAm avirulent (Fig. 2B). This result suggests that CIFA-mediated virulence in this model is solely dependent upon functional fibrinogen binding activity.

### 3.3. Active vaccination with rmCIFA and passive immunization with a functional CIFA monoclonal antibody can ameliorate CIFA-mediated virulence

Mice were immunized with rmCIFA + AlPO4 or AlPO4 alone to determine if vaccination could protect against CIFA-mediated pathogenesis in the *L. lactis*:CIFA systemic infection model. While the majority of animals vaccinated with vehicle succumbed to infection within 24 h, 76% of CIFA-immunized mice survived the challenge (Table 1), suggesting that a vaccine-induced immune response can protect against CIFA-mediated pathogenesis.

To further interrogate CIFA-mediated pathogenesis in the *L. lactis*:CIFA systemic infection model, passive immunization studies were conducted using two CIFA mAbs (12–9 and 15EC6). Although both mAbs bind CIFA, 12–9 is capable of interrupting the binding of *S. aureus* to fibrinogen, as measured by the FBI assay, while 15EC6 cannot [9]. Likewise, passive administration of the functional (i.e., able to inhibit CIFA fibrinogen-binding activity) CIFA mAb 12–9 completely protected mice challenged with *L. lactis*:CIFA, while the nonfunctional mAb 15EC6 did not protect (Fig. 3). Together, these results demonstrate that the lethal phenotype of *L. lactis*:CIFA is dependent upon the ability of CIFA to bind fibrinogen. Disruption of that interaction, either genetically via the Y338A substitution or with the 12–9 mAb that blocks *S. aureus* binding to fibrinogen, abrogates the lethal phenotype.

### 3.4. Defining the preclinical correlate of protection for CIFA-containing vaccines

Using the FBI assay in conjunction with the CIFA-dependent mouse model of infection, we sought to identify a threshold anti-CIFA antibody titer that correlates with protection from the lethal *L. lactis*:CIFA challenge. Those animals that were immunized with rmCIFA and survived challenge with *L. lactis*:CIFA all had high FBI titers (Geometric mean titer (GMT) 1214). Both the AlPO4-treated control animals and the rmCIFA-vaccinated animals that did not survive bacterial challenge had undetectable FBI titers (data not shown). Due to the large disparity in FBI titer, active immunization cannot provide sufficient accuracy to define a protective anti-CIFA threshold. While passive immunization permits a better controlled titration of antibody and an alternative experimental approach to define a protective titer, infusion of anti-CIFA antibody into the animal prior to bacterial challenge requires large amounts of antibody, and is therefore less practical for studies that include multiple animals.

Instead preincubation of the *L. lactis*:CIFA bacteria with functional CIFA antibody concentrations with a range of FBI titers

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**Table 1**

<table>
<thead>
<tr>
<th>Study</th>
<th>Vaccine</th>
<th>% Survival at 24 h</th>
<th>p-value versus control</th>
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<tbody>
<tr>
<td>1</td>
<td>rmCIFA + AlPO4, Vehicle</td>
<td>90</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>rmCIFA + AlPO4, Vehicle</td>
<td>80</td>
<td>0.0028</td>
</tr>
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<td>3</td>
<td>rmCIFA + AlPO4, Vehicle</td>
<td>60</td>
<td>0.0006</td>
</tr>
<tr>
<td>Meta-analysis</td>
<td>rmCIFA + AlPO4, Vehicle</td>
<td>76</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Heterologous expression of *S. aureus* CIFA with functional fibrinogen binding activity is able to confer a lethal phenotype to *L. lactis* in a mouse model of infection. (A) Female BALB/c mice (n = 10/group) were challenged IV with −1 × 10^9 CFU *L. lactis*:CIFA or the *L. lactis*:vector control strain. Survival was monitored twice per day for 7 days. (B) Female BALB/c mice (n = 10/group) were challenged IV with −1 × 10^9 CFU *L. lactis*:CIFA, *L. lactis*:CIFAm, or the *L. lactis*:vector strains. Survival was monitored twice per day for 7 days.

**Fig. 3.** Passive immunization with the functional anti-CIFA mAb 12–9 is protective against lethal *L. lactis*:CIFA challenge in mice. Mice (n = 10–11/group) were passively immunized with 1.5 mg 12–9, 15EC6, or IgG1 isotype control mAb or PBS, and then challenged IV with −1.2 × 10^9 CFU of *L. lactis*:CIFA. Survival was monitored for 7 days.
was chosen as an experimental strategy to identify a protective threshold. In an initial study designed to validate the approach, mice that were challenged with *L. lactis*:ClfA preincubated with 100 μg of the functional 12–9 mAb were able to survive the bacterial challenge (Fig. 4A). In contrast, mice challenged with *L. lactis*:ClfA preincubated with 100 μg of either an isotype control mAb or the non-functional 15EC6 mAb were highly susceptible. There was a dose-dependent relationship between survival of mice in the model and the amount of 12–9 mAb used to precoat the *L. lactis*:ClfA. At levels >50 μg, preincubation with the 12–9 mAb was able to completely protect against CIFA-mediated virulence (Fig. 4B). Collectively, this confirmed the passive immunization data and enabled us to perform further studies using the preincubation model.

Next we performed serial dilutions of mouse polyclonal CIFA antiserum to identify the FBl titer required to confer protection from challenge with precoated *L. lactis*:ClfA. Preincubation with mouse polyclonal sera with a calculated FBl titer of >142 was protective in this model (Table 2). None of the mice challenged with *L. lactis*:ClfA preincubated with polyclonal anti-ClfA serum with calculated FBl titers of <71 survived the infection. In this model, an FBl titer of 142 represents the threshold that correlates with protection.

### 4. Discussion

The work presented here clearly reaffirms CIFA as an important *S. aureus* virulence factor. Ectopic expression of CIFA confers a lethal phenotype to the innocuous organism *L. lactis*. CIFA is a primary fibrinogen-binding factor of *S. aureus*, and the fibrinogen-binding potential of CIFA has been proposed to be critical for its function [4,17,18]. This is supported by the fact that mice lacking the CIFA binding site in the fibrinogen γ chain are more resistant to *S. aureus* sepsis, and *S. aureus* expressing CIFA, which cannot bind fibrinogen, is far less pathogenic in a murine septic arthritis model [18]. We have confirmed that the fibrinogen-binding activity of CIFA is critical for virulence by using CIFA with a single Y338A amino acid substitution in the fibrinogen binding domain. CIAFY338A was unable to bind to fibrinogen, and also was unable to confer the lethal phenotype when expressed in *L. lactis* and used to challenge mice. This finding clearly demonstrates that the fibrinogen-binding capacity of CIFA is the primary mechanism of CIFA-mediated pathogenesis in this model of systemic bacterial infection.

*S. aureus* causes devastating disease, especially in surgical populations and in individuals immunocompromised by obesity, diabetes, or smoking [19–21]. *S. aureus* disease burden and mortality is compounded by the antibiotic resistant clones, such as methicillin-resistant USA300. The prevalence of *S. aureus* disease and the emergence of antibiotic-resistant *S. aureus* highlight the need for an effective *S. aureus* vaccine. Therefore, we were interested to determine if antibodies directed against CIFA could confer protection. Preincubation with either a functional CIFA monoclonal antibody or murine polyclonal sera was sufficient to protect in the *L. lactis*:CIFA systemic infection model. This implies that antibodies can abrogate the CIFA-mediated virulence that is dependent upon CIFA fibrinogen binding. Importantly, only polyclonal serum which contained functional antibodies, as measured by the ability to inhibit fibrinogen binding in the FBl assay, could protect in the preincubation systemic infection model. Despite routine environmental exposure to *S. aureus*, humans generally do not appear to have circulating functional anti-CIFA antibodies that inhibit binding of whole CIFA-expressing bacteria to fibrinogen [9]. Not surprisingly, therefore, while many human subjects have CIFA binding antibodies, and some individuals have antibodies that prevent the binding of CIFA protein to fibrinogen, most human subjects have functional CIFA antibody titers below the limit of detection in the FBl assay [9,22]. Similarly, unimmunized mice do not have detectable FBl titers, and preincubation of *L. lactis*:CIFA bacteria using serum samples with FBl titers <142 does not protect in the systemic challenge model.

The fact that natural exposure does not generally induce functional anti-CIFA antibodies in humans begged the question as to whether vaccination would be able to induce a protective anti-CIFA immune response. Hawkins et al had previously shown that immunization of humans with a CIFA-containing vaccine elicited a functional response against CIFA [9]. We immunized mice with CIFA and showed that active vaccination was capable of protecting mice in the *L. lactis*:CIFA systemic infection model. Thus, vaccination may overcome the inability of natural exposure to induce a functional anti-CIFA immune response, and this response can be measured by the FBl assay. These results support the use of the FBl assay as a preclinical measure of protection and suggest that the assay may be useful in defining a clinical correlate for protection based on functional CIFA antibodies for CIFA-containing vaccines.

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**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Calculated FBl titer</th>
<th>Surviving animals/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% CIFA mouse serum</td>
<td>284</td>
<td>4/4</td>
</tr>
<tr>
<td>60% CIFA mouse serum</td>
<td>213</td>
<td>5/5</td>
</tr>
<tr>
<td>40% CIFA mouse serum</td>
<td>142</td>
<td>5/5</td>
</tr>
<tr>
<td>20% CIFA mouse serum</td>
<td>71</td>
<td>0/4</td>
</tr>
<tr>
<td>80% Normal Mouse Serum</td>
<td>40</td>
<td>0/5</td>
</tr>
<tr>
<td>50 mcg 12–9</td>
<td></td>
<td>5/5</td>
</tr>
</tbody>
</table>
Antibodies to ClfA have been tested as a stand-alone therapy in preclinical models and, while there was some indication of amelioration of S. aureus disease, ultimately these approaches have yet to be validated in the clinic. It should be noted that one of those approaches, Veronate, consisted of purified total IgG from humans with high ClfA binding antibody titers resulting from natural exposure. As natural exposure does not generally induce functional anti-ClfA antibodies in humans, it is possible that this product lacked efficacy due to the absence of sufficient amounts of functional antibody.

Active vaccination, unlike passive immunotherapy, can induce the production of functional antibodies to meet the demand posed by an infection. Prophylactic vaccination has the ability to target the relatively small inocula of bacteria that initiate an infection, rather than trying to overcome a fulminant infection. Therefore a prophylactic vaccine against S. aureus, especially one containing multiple antigens, has a greater likelihood of success than a passive immunotherapeutic approach. Passive immunotherapies composed of well-defined functional antibodies directed against well-understood targets remain valuable to ameliorate disease in emergent situations where there is no time to induce a protective immune response through vaccination. Here we have shown that active vaccination can protect against ClfA-mediated virulence in the L. lactis:ClfA systemic infection model, and that protection correlated with the induction of a functional antibody response, as measured by the FBL assay. This work is a significant advance towards potentially identifying a correlative of protection for ClfA that could be evaluated in clinical efficacy trials with ClfA-containing vaccines.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.08.029.

References