Fibrin(ogen) engagement of *S. aureus* promotes the host antimicrobial response and suppression of microbe dissemination following peritoneal infection

Oscar Negrón, Woosuk S. Hur, Joni Prasad, David S. Paul, Sarah E. Rowe, Jay L. Degen, Sara R. Abrahams, Silvio Antoniak, Brian P. Conlon, Wolfgang Bergmeier, Magnus Höök, Matthew J. Flick

1 Department of Pathology and Laboratory Medicine, UNC Blood Research Center, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 2 Division of Experimental Hematology, Cincinnati Children’s Hospital Medical Center and University of Cincinnati School of Medicine, Cincinnati, Ohio, United States of America, 3 Department of Biochemistry, UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 4 Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States of America, 5 Center of Infectious and Inflammatory Diseases, Texas A&M Health Sciences Center, Houston, Texas, United States of America

* matthew_flick@med.unc.edu

Abstract

The blood-clotting protein fibrin(ogen) plays a critical role in host defense against invading pathogens, particularly against peritoneal infection by the Gram-positive microbe *Staphylococcus aureus*. Here, we tested the hypothesis that direct binding between fibrin(ogen) and *S. aureus* is a component of the primary host antimicrobial response mechanism and prevention of secondary microbe dissemination from the peritoneal cavity. To establish a model system, we showed that fibrinogen isolated from FibγΔ5 mice, which express a mutant form lacking the final 5 amino acids of the fibrinogen γ chain (termed fibrinogenγΔ5), did not support *S. aureus* adherence when immobilized and clumping when in suspension. In contrast, purified wildtype fibrinogen supported robust adhesion and clumping that was largely dependent on *S. aureus* expression of the receptor clumping factor A (CfA). Following peritoneal infection with *S. aureus* USA300, FibγΔ5 mice displayed worse survival compared to WT mice coupled to reduced bacterial killing within the peritoneal cavity and increased dissemination of the microbes into circulation and distant organs. The failure of acute bacterial killing, but not enhanced dissemination, was partially recapitulated by mice infected with *S. aureus* USA300 lacking CfA. Fibrin polymer formation and coagulation transglutaminase Factor XIII each contributed to killing of the microbes within the peritoneal cavity, but only elimination of polymer formation enhanced systemic dissemination. Host macrophage depletion or selective elimination of the fibrin(ogen) β2-integrin binding motif both compromised local bacterial killing and enhanced *S. aureus* systemic dissemination, suggesting fibrin polymer formation in and of itself was not sufficient to retain *S. aureus* within the peritoneal cavity. Collectively, these findings suggest that following peritoneal infection, the binding of *S. aureus* to stabilized fibrin matrices promotes a local, macrophage-mediated...
antimicrobial response essential for prevention of microbe dissemination and downstream host mortality.

Author summary

The Gram-positive bacterium Staphylococcus aureus (S. aureus) produces a number of soluble and surface-associated proteins that bind the host coagulation protein fibrinogen. The contribution of fibrinogen-S. aureus binding through the fibrinogen receptor clumping factor A (ClfA) in peritoneal infection has not been defined. Elimination of the binding motif on fibrinogen for ClfA or deletion of ClfA from S. aureus significantly reduced S. aureus-fibrinogen binding and bacterial clumping in solution. In a mouse model of peritonitis, loss of these activities resulted in diminished bacterial killing, increased bacterial dissemination, and worsened host survival. Although fibrin polymer formation and fibrin(ogen)-macrophage binding are mechanistically linked to the local antimicrobial response, fibrin formation in and of itself is not sufficient to suppress microbe dissemination. These discoveries have identified important components of the fibrin(ogen)-dependent host antimicrobial response against S. aureus, providing further understanding of this physiological response to infection which could uncover potential therapeutic strategies for peritonitis patients.

Introduction

Staphylococcus aureus (S. aureus) is a common, Gram-positive bacterium that colonizes 20–80% of healthy adults [1]. It is the causative agent for a variety of illnesses ranging from minor skin infections to more serious and life-threatening conditions such as bacteremia, sepsis, infective endocarditis, and pneumonia [2]. S. aureus is frequently identified in both community- and hospital-acquired settings [3]. Even with appropriate treatment strategies, S. aureus bacteremia has a 30-day mortality rate of 20–40% [4], an issue that is further complicated by the emergence of methicillin and vancomycin resistant (M.R.S.A. and V.R.S.A.) strains of S. aureus [5]. Thus, there is a need to better understand the mechanisms of pathogenesis of S. aureus in order to develop improved treatment strategies that do not strictly rely on antibiotics.

S. aureus expresses multiple virulence factors that allow the bacterium to engage and manipulate components of the host coagulation system, including a variety of proteins that directly bind fibrin(ogen). Specifically, S. aureus produces a family of virulence factors called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), such as the fibrin(ogen) binding protein Clumping factor A (ClfA). ClfA binds to fibrin(ogen) at the carboxy-terminal domain of the fibrinogen γ chain and mediates bacterial clumping in host plasma and bacterial adhesion to fibrin(ogen)-coated surfaces [6]. ClfA has been identified as an important factor of S. aureus virulence in animal models of bacteremia, septic arthritis and endocarditis through fibrin(ogen)-dependent and independent mechanisms [7–11].

Although S. aureus utilizes host hemostatic factors like fibrin(ogen) to support its virulence, the host hemostatic system plays an important role in antimicrobial defense. Indeed, fibrin (ogen) can function as a protective barrier, trapping invading microbes in matrices to inhibit bacterial growth and dissemination through the host [6,12,13]. Fibrin(ogen) also functions as a modulator of the host immune response. Neutrophils and macrophages expressing the
integrin receptor αMβ2 can bind fibrin(ogen), leading to activation and initiation of antimicrobial functions like phagocytosis, production of reactive oxygen species (ROS), and pro-inflammatory cytokine production [14–18]. For peritoneal S. aureus infections, fibrinogen(ogen) was shown to play a pivotal role in host defense. Studies of fibrinogen-deficient (Fib−/−) mice revealed that these animals display poor survival and an inability to clear bacteria from the peritoneal cavity following acute infection [19]. Here, we tested the hypothesis that S. aureus binding to fibrinogen(ogen) via ClfA is a key feature of the antimicrobial mechanism following peritoneal infection. Further, we determined the impact of platelets, fibrin polymer formation, fibrin crosslinking, and the fibrinogen(macrophage) response to the dissemination of bacteria from the peritoneum and the establishment of systemic disease.

Results

The fibrinogen γ-chain AGDV motif is required for S. aureus adhesion to immobilized fibrinogen and for clumping in a fibrinogen solution in vitro

The C-terminal portion of the fibrinogen γ-chain encodes a motif for interaction of S. aureus through the receptor ClfA, as well as other S. aureus fibrinogen binding proteins such as fibronectin-binding protein A and B (Fnbp-A and Fnbp-B) [11,20–22]. Fib55 mice express a mutant form of fibrinogen lacking the final AGDV motif. Adhesion and clumping experiments were performed using fibrinogen purified from wildtype (fibrinogenWT) and Fibγ55 mice (fibrinogenγAS) to characterize the impact of the loss of the AGDV motif on fibrinogen-S. aureus interactions. WT S. aureus USA300 grown to stationary phase bound to immobilized fibrinogenWT in a dose dependent manner whereas fibrinogenγAS did not support bacterial adhesion at any coating concentration (Fig 1A). Genetic elimination of ClfA from the bacterial surface (ClfA−) resulted in loss of bacterial binding to both immobilized fibrinogen species (Fig 1B). Notably, analysis of ClfA− pclfa S. aureus, a complemented ClfA- USA300 strain in which expression of ClfA was reconstituted, restored binding to fibrinogenWT but not to fibrinogenγAS (Fig 1C). In assays evaluating solution phase S. aureus clumping, fibrinogenWT supported clumping in a dose dependent manner but no clumping was observed with fibrinogenγAS (Fig 1D). As expected, ClfA− S. aureus USA300 did not support clumping with fibrinogenWT and fibrinogenγAS (Fig 1E), whereas ClfA− pclfa S. aureus USA300 displayed a clumping pattern identical to WT S. aureus USA300 (Fig 1F).

S. aureus produces other fibrinogen-binding proteins, including Fnbp-A and Fnbp-B that engage fibrin(ogen) via the fibrinogen γ-chain [6]. However, while ClfA is expressed throughout the entire growth cycle of S. aureus, Fnbp-A and Fnbp-B are expressed predominantly during the exponential growth phase [23–26]. To determine a potential role for these additional fibrinogen-binding proteins, we performed adhesion and clumping experiments with S. aureus USA300 grown to exponential phase in the presence of fibrinogenWT or fibrinogenγAS. Similar to S. aureus at stationary phase, exponential phase WT S. aureus USA300 bound immobilized fibrinogenWT in a dose dependent manner whereas fibrinogenγAS did not support bacterial adhesion at any coating concentration (Fig 1G). However, exponential phase ClfA− S. aureus USA300 was able to bind fibrinogenWT, but fibrinogenγAS did not support bacterial adhesion (Fig 1H). ClfA− pclfa S. aureus displayed a pattern identical to WT S. aureus (Fig 1I). When evaluating S. aureus clumping, exponential phase WT S. aureus USA300 showed reduced clumping in the presence of fibrinogenWT when compared to stationary phase bacteria and no clumping was observed in the presence of fibrinogenγAS (Fig 1J). Exponential phase ClfA− S. aureus USA300 did not support clumping with either of the fibrinogens analyzed (Fig 1K), whereas ClfA− pclfa S. aureus displayed a pattern akin to WT S. aureus (Fig 1L).
The fibrin(ogen) AGDV binding motif is required for both *S. aureus* adhesion to immobilized fibrinogen and clumping in fibrinogen solution. Adhesion of stationary phase (A) WT *S. aureus* USA300, (B) ClfA- *S. aureus* USA300, and (C) ClfA<sup>pclfa</sup> *S. aureus* USA300 to immobilized fibrinogen<sup>WT</sup> or fibrinogen<sup>Δ5</sup>. Clumping of stationary phase (D) WT *S. aureus* USA300, (E) ClfA- *S. aureus* USA300, or (F) ClfA<sup>pclfa</sup> *S. aureus* USA300 in solutions containing fibrinogen<sup>WT</sup> or fibrinogen<sup>Δ5</sup>. Adhesion of exponential phase (G) WT *S. aureus* USA300, (H) ClfA- *S. aureus* USA300, or (I) ClfA<sup>pclfa</sup> *S. aureus* USA300 to immobilized fibrinogen<sup>WT</sup> or fibrinogen<sup>Δ5</sup>. Clumping of exponential phase (J) WT *S. aureus* USA300, (K) ClfA- *S. aureus* USA300, or (L) ClfA<sup>pclfa</sup> *S. aureus* USA300 in solutions containing fibrinogen<sup>WT</sup> or fibrinogen<sup>Δ5</sup>. Data is derived from *N* = 3 replicates per fibrinogen concentration per group and presented as mean absorbance (for adhesion experiments) or the inverse of mean absorbance (for clumping experiments) ± SEM. Statistical significance was determined by 2-way ANOVA with Sidak’s multiple comparisons test where + = <0.05, * = <0.01, ** = <0.001, and *** = <0.0001.

https://doi.org/10.1371/journal.ppat.1010227.g001
findings suggest that the fibrinogen γ-chain AGDV motif supports fibrinogen-\textit{S. aureus} interactions, with a major fraction of the binding occurring through ClfA.

**Elimination of the γ-chain AGDV motif results in decreased survival following \textit{S. aureus} peritonitis**

To determine the role of \textit{S. aureus}-fibrinogen interactions on host survival following peritoneal infection, cohorts of Fibγ\textsubscript{WT} and Fibγ\textsubscript{Δ5} mice were given an intraperitoneal (i.p.) infection with \(\sim 5 \times 10^8\) CFUs of WT \textit{S. aureus} USA300 and animal survival was monitored. Fibγ\textsubscript{Δ5} showed a significant decrease in survival with only 40\% of mice remaining after a 1-week observation period whereas Fibγ\textsubscript{WT} mice were largely protected (Fig 2A). These data suggest that fibrin(ogen)-\textit{S. aureus} interactions support a host defense mechanism important for host survival following peritonitis.

The accumulation of fibrinogen and other plasma components entering the peritoneal cavity following infection was next analyzed. In an Evans Blue vascular leak assay, Fibγ\textsubscript{WT} and Fibγ\textsubscript{Δ5} each had low levels of retrievable Evans Blue dye from the peritoneal cavity in the absence of infection (Fig 2B). In contrast, mice challenged with a peritoneal \textit{S. aureus} infection

---

**Fig 2.** Fibγ\textsubscript{Δ5} mice display decreased survival following \textit{S. aureus} peritonitis. (A) Kaplan-Meyer log-rank analysis of Fibγ\textsubscript{WT} (n = 20) and Fibγ\textsubscript{Δ5} (n = 20) mice following i.p. infection with \(4.7 \times 10^8\) CFUs of \textit{S. aureus} USA300. (B) Evans Blue vascular leak assay on Fibγ\textsubscript{WT} and Fibγ\textsubscript{Δ5} mice following i.p. injection with PBS or \(\sim 1 \times 10^9\) CFU of \textit{S. aureus} USA300. Fibrinogen ELISA on lavage fluid (C) and plasma (D) following 1 hour i.p. infection with \textit{S. aureus} USA300. Data are presented as mean ± SEM and statistical significance was determined by 2-way ANOVA with Sidak’s multiple comparisons test.

https://doi.org/10.1371/journal.ppat.1010227.g002
had quantifiably higher levels of Evans Blue retrievable from the peritoneal cavity 1 hour after infection with no genotype-dependent differences detected (Fig 2B). ELISA assays for fibrinogen were also performed on peritoneal lavage fluid collected from FibγWT and FibγAS mice uninfected as well as 1 hour after i.p. injection with ~1x10⁹ CFUs of S. aureus USA300. Peritoneal lavage fluid from uninfected mice contained very little fibrinogen, but the lavage fluid from infected animals contained readily detectable concentrations of fibrinogen that were comparable between both genotypes (Fig 2C). Plasma fibrinogen levels were similar between both genotypes in uninfected mice as previously reported [27], and were modestly lower in each genotype 1 hour after infection (Fig 2D). Collectively, these findings indicate that the observed survival phenotypes were not due to a failure of plasma proteins, including fibrinogen, to enter the peritoneal cavity of infected FibγAS mice.

**FibγAS mice show increased S. aureus CFUs in the peritoneal cavity following infection**

Previous studies identified a rapid, local fibrin-driven antimicrobial activity against S. aureus peritoneal infection [19]. To determine whether fibrinogen-S. aureus interactions are a component of that host defense mechanism, cohorts of FibγWT and FibγΔ5 mice were challenged with an i.p. infection with ~5x10⁸ CFUs of WT S. aureus USA300 and peritoneal lavage fluid analyzed 1 or 4 hours after infection. WT mice rapidly eliminated ~99% of the initial inoculum within 1 hour in a manner similar to previous findings [19] whereas FibγAS mice showed significantly higher bacterial CFUs following infection (Fig 3A). Analysis of cytospin images of the lavage fluid showed excessive free-floating bacteria in FibγAS mice while FibγWT mice had little to no free-floating bacteria (Fig 3B). Interestingly, at 4 hours after infection, no significant differences in S. aureus CFUs in the peritoneal lavage fluid were observed between genotypes (Fig 3C). Cytospin images showed little to no free-floating bacteria in the peritoneal lavage fluid of FibγWT and FibγAS mice at 4 hours after infection (Fig 3D).

No differences in total host cells retrievable from the peritoneal cavity were observed between genotypes at 1 hour (Fig 3E) post-infection; however, a modest but statistically significant decrease in cell numbers was observed in FibγAS mice at 4 hours (Fig 3F) post-infection. FibγAS had marginally higher macrophages and lower lymphocytes relative to FibγWT mice within the lavage fluid at 1 hour after infection (Fig 3G). At 4 hours after infection, neutrophils were the most prevalent cell type as expected (Fig 3H), and FibγAS had more macrophages but lower neutrophils relative to FibγWT mice (Fig 3H). Quantitative assessment indicated that lavage fluid harvested from FibγAS contained significantly higher numbers of host cells with internalized bacteria relative to FibγWT mice at both 1 hour and 4 hours after infection (Figs 3I and 3J). Collectively, these data suggest that the reduction in bacterial clearance observed in FibγAS mice is not due to a genotype-dependent difference in host cells within the peritoneal cavity nor a failure of the peritoneal cells to phagocytose bacteria.

**Elimination of S. aureus-fibrinogen γ-chain interactions results in rapid bacterial dissemination from the peritoneal cavity**

We also sought to determine whether the early, local failure in bacterial clearance displayed by FibγAS mice was associated with enhanced bacterial dissemination. At 1 hour after peritoneal infection, the S. aureus burden was significantly higher in the blood as well as heart and lung (e.g., organs distant from the peritoneal cavity) of FibγAS mice compared to FibγWT mice (Fig 4A, 4B and 4C). At 4 hours after infection, higher levels of CFUs in blood, heart, and lung tissues were also observed in FibγAS mice relative to FibγWT mice (Fig 4D, 4E and 4F). To determine if the increased overall bacterial burden observed in FibγAS mice resulted in systemic
Fig 3. FibγΔ5 mice display increased S. aureus CFUs in the peritoneal cavity following infection. (A) Total bacterial CFUs in peritoneal lavage fluid of FibγWT and FibγΔ5 mice 1 hour after i.p. infection with 5x10^8 CFUs WT USA300 S. aureus. (B) Representative images of cytospin preparations of peritoneal lavage fluid collected from FibγWT and FibγΔ5 mice 1hr after i.p. infection with 5x10^8 CFUs USA300 S. aureus. Note the presence of extensive free-floating bacteria in FibγΔ5 mice compared to FibγWT. (C) Total CFU in the peritoneal lavage fluid of FibγWT and FibγΔ5 mice 4 hours after i.p. infection with 4x10^8 CFUs of WT USA300 S. aureus. (D) Representative images of cytospin preparations of peritoneal lavage fluid collected from FibγWT and FibγΔ5 mice 4 hours after infection. CFU data is presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test. Dashed horizontal lines indicate the infection dose. Images were captured with 40x objective with scale bar representing 20 μm. Total cell counts from peritoneal lavage fluid 1 hour (E) and 4
hours (F) after infection with USA300 S. aureus. Data are presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test. Differential cell counts from peritoneal lavage fluid 1 hour (G) and 4 hours (H) after infection. Data are presented as mean ± SEM and statistical significance was determined by 2-way ANOVA with Šidák’s multiple comparisons test. Analysis of total cells with internalized bacteria 1 hour (I) and 4 hours (J) after infection. Data are presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test.

https://doi.org/10.1371/journal.ppat.1010227.g003

evidence of tissue damage to the host at the 4-hour time point, markers of coagulation activation and tissue damage were analyzed. There were no significant differences in plasma thrombin-antithrombin complexes and D-dimer in mice of any genotype (S1A and S1B Fig). No differences in plasma lactate dehydrogenase (LDH), creatine kinase (CK), or alanine aminotransferase (ALT) were detected based on genotype or infection (S1C, S1D and S1E Fig). A modest but statistically significant difference in cardiac troponin I was detected in infected FibγΔ5 mice relative to infected FibγWT mice, consistent with the higher S. aureus bacterial burdens in the hearts of those animals (S1F Fig).

Fig 4. FibγΔ5 mice display increased S. aureus dissemination to organ tissues. Total CFUs in (A) blood, (B) heart and (C) lung of FibγWT and FibγΔ5 mice 1 hour after i.p. infection with 5×10⁸ CFUs of WT S. aureus USA300. Total CFUs in (D) blood, (E) heart and (F) lung of FibγWT and FibγΔ5 mice 4 hours after i.p. infection with 4×10⁸ CFUs of WT S. aureus USA300. Data are represented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test.

https://doi.org/10.1371/journal.ppat.1010227.g004
Elimination of platelets but not platelet-derived protease-activated receptor-4 results in increased retrievable S. aureus in the peritoneal cavity

In addition to supporting binding to various S. aureus fibrinogen binding proteins, the fibrinogen γ-chain AGDV motif also supports binding to platelet integrin αIIbβ3. Thus, differences in S. aureus infection observed in the Fibrγ−/− mice could be linked to platelets or platelet function. To determine a potential contribution of platelets in S. aureus peritonitis, mice that received a sham injection or a platelet-depleting antibody (Fig 5A) were challenged for 1 hour with 8x10^8 CFUs of WT S. aureus USA300 by intraperitoneal injection. Platelet-depleted mice showed a modest but significant increase in S. aureus CFUs in peritoneal lavage fluid (Fig 5B). However, no significant differences were observed in CFUs quantified from blood (Fig 5C), heart (Fig 5D) and lung (Fig 5E). Notably, there were no differences in the fibrinogen accumulation in the lavage fluid (S2A Fig) nor plasma fibrinogen levels between sham-treated and platelet-depleted mice (S2B Fig). The total number of host leukocytes retrievable from the peritoneal cavity were similar between both groups (Fig 5F).

Given that thrombin activity was previously documented as a component of the mechanism of fibrin-dependent S. aureus clearance from the peritoneal cavity [28], we next determined if protease-activated receptor-4 (PAR-4) mediated platelet activation was linked to changes in S. aureus clearance following peritoneal infection. Mice with a platelet-specific deletion in PAR-4 (i.e., Pf4-Cre/PAR4fl/fl) were analyzed. Pf4-Cre+/PAR4fl/fl and PAR4fl/fl controls were challenged for 1 hour with 6.6x10^8 CFUs of WT S. aureus USA300 via i.p. injection. There were no observable differences in S. aureus CFUs in peritoneal lavage fluid (Fig 5G), blood (Fig 5H), heart (Fig 5I) and lung (Fig 5J). The total number of host leukocytes retrievable from the peritoneal cavity were also similar between both groups (Fig 5K). Collectively, these data suggest that platelets make a modest, but statistically significant, PAR4-independent contribution to the local host antimicrobial response against S. aureus peritonitis but elimination of platelets does not play a major role in promoting bacterial dissemination.

Deletion of ClfA from S. aureus USA300 results in a reduction of bacterial clearance with an increased initial, but not sustained dissemination/accumulation of bacteria in distant organs

Our in vitro findings indicated that ClfA plays a major role in S. aureus adhesion and clumping to fibrinogen. To determine if ClfA is also critical for the host antimicrobial response and suppression of dissemination following peritoneal infection, WT mice were infected with WT or ClfA− S. aureus USA300 by intraperitoneal injection. Similar to what was observed in Fibrγ−/− mice, mice challenged with ClfA− S. aureus USA300 had increased bacterial CFUs in the peritoneal cavity 1 hour after infection relative to mice infected with WT S. aureus (Fig 6A). Notably, the accumulation of fibrinogen within the peritoneal cavity was equivalently increased in mice infected with either WT or ClfA− S. aureus USA300 compared to uninfected mice (S2C Fig) with no significant changes in circulating fibrinogen (S2D Fig). In addition, mice infected with ClfA− S. aureus USA300 displayed a modest, but statistically significant increase in bacterial dissemination/accumulation into the blood (Fig 6B), heart (Fig 6C) and a trend to higher CFUs in the lung tissue (Fig 6D) compared to mice infected with WT S. aureus. Mice infected with WT or ClfA− S. aureus showed similar numbers of host leukocytes retrievable from the peritoneal cavity 1 hour after infection (Fig 6E). Differential cell counts from peritoneal lavage fluid revealed no significant differences in immune cell types in mice infected with WT or ClfA− S. aureus (Fig 6F). However, WT animals infected with ClfA− S. aureus showed higher numbers of host cells with internalized bacteria 1 hour after infection (Fig 6G). Notably, infection of WT mice with ClfA− S. aureus resulted in local retrievable CFUs (S3A Fig) and...
disseminated bacteria (S3B–S3D Fig) in numbers virtually identical to WT *S. aureus*. Furthermore, host immune cell numbers (S3E Fig) were similar to mice infected for 1 hour with WT *S. aureus*. Collectively, these findings suggest that differences in local and systemic CFUs in mice infected with ClfA- *S. aureus* were due to the loss of ClfA itself and not to unintended secondary changes in the ClfA- *S. aureus* USA300 strain.

WT mice were also infected with WT or ClfA- *S. aureus* USA300 by intraperitoneal injection for 4 hours. Here, no differences in CFUs in peritoneal lavage fluid were observed.
Deletion of ClfA from *S. aureus* USA300 results in increased *S. aureus* CFUs in the peritoneal cavity and a transient increase in CFUs the bloodstream and distant organs. Total live bacteria in (A) lavage, (B) blood, (C) heart and (D) Lung of WT mice 1 hour after i.p. infection with WT (1x10^9 CFUs) or ClfA- (7.8x10^8 CFUs) *S. aureus* USA300. (E) Total host cells, (F) host cell differentials, and (G) and percentage of host cells with internalized bacteria in lavage fluid at 1 hour after infection. Total live bacteria in (H) lavage, (I) blood, (J) heart and (K) lung of WT mice 4 hours after i.p. infection with WT (1.6x10^9 CFUs) or ClfA- (1.2x10^9 CFUs) *S. aureus* USA300. (L) Total host cells, (M) host cell differentials, and (N) and percentage of host cells with internalized bacteria in lavage fluid at 1 hour after infection. CFU data are presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test. Host cell counts and percentages are presented as mean ± SEM with statistical significance determined by 2-way ANOVA with Šidák’s multiple comparisons test. Data on cells with internalized bacteria are presented as mean ± SEM and statistical significance was determined by Mann-Whitney U-test. Dashed horizontal lines indicate the infection dose.

Fig 6. Deletion of ClfA from *S. aureus* USA300 results in increased *S. aureus* CFUs in the peritoneal cavity and a transient increase in CFUs the bloodstream and distant organs. Total live bacteria in (A) lavage, (B) blood, (C) heart and (D) Lung of WT mice 1 hour after i.p. infection with WT (1x10^9 CFUs) or ClfA- (7.8x10^8 CFUs) *S. aureus* USA300. (E) Total host cells, (F) host cell differentials, and (G) and percentage of host cells with internalized bacteria in lavage fluid at 1 hour after infection. Total live bacteria in (H) lavage, (I) blood, (J) heart and (K) lung of WT mice 4 hours after i.p. infection with WT (1.6x10^9 CFUs) or ClfA- (1.2x10^9 CFUs) *S. aureus* USA300. (L) Total host cells, (M) host cell differentials, and (N) and percentage of host cells with internalized bacteria in lavage fluid at 1 hour after infection. CFU data are presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test. Host cell counts and percentages are presented as mean ± SEM with statistical significance determined by 2-way ANOVA with Šidák’s multiple comparisons test. Data on cells with internalized bacteria are presented as mean ± SEM and statistical significance was determined by Mann-Whitney U-test. Dashed horizontal lines indicate the infection dose.

https://doi.org/10.1371/journal.ppat.1010227.g006
between mice infected with WT or ClfA- *S. aureus*, consistent with what was observed in the Fibγ55 mice (Fig 6H). However, while Fibγ55 mice showed increased bacterial burdens in blood and organ tissues 4 hours after infection, WT mice infected with ClfA- *S. aureus* had similar CFUs in blood, heart, and lung tissue relative to mice infected with WT *S. aureus* (Fig 6L–6K). At this time point, no difference in total retrievable cells from the peritoneal cavity were found (Fig 6L). A modest, but statistically significant, difference in immune cell types was observed where mice infected with ClfA- *S. aureus* showed higher macrophage and lower neutrophil numbers when compared to mice infected with WT *S. aureus* (Fig 6M). Similar to findings at 1 hour, WT animals infected with ClfA- *S. aureus* had higher numbers of host cells with internalized bacteria at 4 hours after infection (Fig 6N). Collectively, these findings suggest that ClfA-fibrinogen binding significantly contributes to the acute host antimicrobial response in the peritoneal cavity and early dissemination. However, eliminating ClfA does not result in a sustained increase in bacterial accumulation within distant organs.

**Fibrin-macrophage driven host antimicrobial function limits dissemination, but fibrin matrix formation alone is not sufficient to suppress systemic spread of *S. aureus* from the peritoneal cavity**

To assess whether fibrinogen is sufficient for limiting bacterial dissemination, FibAEK mice that express fibrinogen with a mutated Aα chain that renders it insensitive to thrombin cleavage and downstream polymer formation were infected with 8.8×10^8 CFUs of WT *S. aureus* USA300 for 1 hour. As previously observed [29], FibAEK mice had significantly higher CFUs in peritoneal lavage fluid when compared to FibWT mice (Fig 7A). Notably, FibAEK mice also showed a significant increase in the bacterial CFUs in blood and lung, with a trend towards higher CFUs in the heart (Fig 7B, 7C and 7D). Total host cells retrieved in lavage showed similar numbers in WT and FibAEK mice (Fig 7E). Here, we also show for the first time that the fibrin crosslinking by transglutaminase Factor XIII (FXIII) contributes to host local antimicrobial function in the peritoneal cavity. Mice deficient in the catalytic A subunit of FXIII (i.e., F13α−/− mice) had significantly higher CFUs in peritoneal lavage when compared to WT mice 1 hour after intraperitoneal infection with 1×10^9 CFUs of WT *S. aureus* USA300 (Fig 7F). However, there were no differences in bacterial dissemination to the blood, heart, or lung (Fig 7G, 7H and 7I). There were also similar numbers of host cells retrieved from lavage in WT and F13α−/− mice (Fig 7J). Collectively, these data indicate that both fibrin polymer formation and FXIIIa-crosslinking play important roles in the acute host antimicrobial response, but whereas loss of polymer formation enhances dissemination, loss of FXIII crosslinking does not increase *S. aureus* escape from the peritoneal cavity.

Finally, to ascertain whether fibrin formation in and of itself offers protection to the host by retaining bacteria within the peritoneal cavity and thus preventing dissemination, macrophage depletion studies were performed. Previous studies revealed that macrophages play an important role in eliminating *S. aureus* within the peritoneal cavity [19,30]. WT mice were given PBS control or Clodronate liposomes 24 hours before infection with 9×10^8 CFUs of WT *S. aureus* USA300. Consistent with published data, macrophage depletion resulted in a reduction of bacterial clearance from the peritoneal cavity 1 hour after infection (Fig 7K). Here, we show that macrophage depletion also resulted in significantly increased bacterial dissemination into circulation (Fig 7L) and lung tissue (Fig 7N) and trends towards higher CFUs in heart tissue (Fig 7M). As expected, there was a reduction in retrievable total host cells in clodronate-treated mice (Fig 7O). To extend these findings, Fibγ390−396A mice with a mutation in the fibrinogen γ chain that eliminates the leukocyte integrin αMβ2 binding motif were analyzed. Previous studies documented that fibrinogen γ390−396A polymerizes identical
Fig 7. Fibrin-macrophage driven host antimicrobial function limits dissemination, but fibrin matrix formation alone is not sufficient to suppress systemic spread of *S. aureus* out of the peritoneal cavity. Total live bacteria in (A) lavage fluid, (B) blood, (C) heart and (D) lung and total host cell counts (E) from WT and Fib$^{A2K}$ mice 1 hr after infection with 8.8x10$^8$ CFUs of WT *S. aureus* USA300. Total live bacteria in lavage fluid (F), blood (G), heart (H) and lung (I) and total host cell counts (J) from WT and FXIII$^{−/−}$ mice 1 hr after infection with 1x10$^9$ CFUs of WT *S. aureus* USA300. Total live bacteria in lavage fluid (K), blood (L), heart (M) lung (N) and total host cell count (O) from WT mice treated with PBS or Clodronate liposomes 1 hr after infection with 9x10$^8$ CFUs of WT *S. aureus* USA300. Total live bacteria in lavage fluid (P), blood (Q), heart (R) and lung (S) and total host cell counts (T) from WT and Fib$^{γ390−396A}$ mice 1 hr after infection with 6.7x10$^8$ CFUs of WT *S. aureus* USA300. Data are presented as mean ± SEM and statistical significance was determined by Mann-Whitney u-test.

https://doi.org/10.1371/journal.ppat.1010227.g007
to WT fibrinogen but does not support fibrin-mediated macrophage binding and activation [31]. Following infection with 6.7x10⁸ CFUs of WT *S. aureus* USA300, Fibγ³⁹⁰-³⁹⁶A mice displayed significantly increased CFUs in peritoneal lavage (Fig 7P). Importantly, Fibγ³⁹⁰-³⁹⁶A mice also displayed a trend towards increased CFUs in the blood (Fig 7Q) and significantly increased CFUs in the heart and lung (Fig 7R and 7S, respectively), relative to infected WT mice. Analysis of total host cells in the peritoneal cavity showed no differences between WT and Fibγ³⁹⁰-³⁹⁶A mice, again suggesting that the observed differences in bacterial clearance and dissemination were not due to changes in leukocyte numbers in the peritoneal cavity (Fig 7T). Collectively, these data suggest that fibrin polymer formation in the absence of the antimicrobial immune response is not sufficient for suppressing *S. aureus* dissemination following peritoneal infection.

**Discussion**

Fibrin(ogen) is a centerpiece and trigger of a potent host *S. aureus* killing mechanism in the peritoneal cavity. Previous studies showed that whereas WT mice are able to eliminate ~99% of an initial peritoneal *S. aureus* infection within 15 minutes, Fib⁻/⁻ mice fail to eliminate the bacteria and rapidly succumb to the infection [19]. Here, we provide evidence that fibrin (ogen) binding to the microbe itself is a key component of the host bacterial killing mechanism. Elimination of the fibrinogen carboxy-terminal γ-chain AGDV motif (i.e., using FibγΔ⁵ mice) compromised bacterial killing. The number of retrievable *S. aureus* CFUs from the peritoneal cavity as well as the number of FibγΔ⁵ mice that succumbed to peritoneal *S. aureus* infection was significantly higher than those observed in WT mice, suggesting that elimination of *S. aureus*-fibrin(ogen) binding reduces the efficiency of the antimicrobial response. *S. aureus*-fibrin(ogen) binding was important for suppression of bacterial dissemination from the peritoneal cavity. Specifically, FibγΔ⁵ mice displayed higher levels of local *S. aureus* CFUs following infection, and robust levels of *S. aureus* in the blood and distant organ systems at 1 and 4 hours after peritoneal infection. Previous studies indicated that bacterial colonization into organs is accompanied by elevated markers of tissue damage in circulation at later time points after infection (e.g., 24 and 48 hours) [11]. Here, analyses at 4 hours after infection showed a modest, but statistically significant increase in cardiac troponin I was detected in FibγΔ⁵ mice. A finding that suggests cardiac activity may be particularly sensitive to the systemic spread of *S. aureus* microbes and that loss of heart function may be the basis for the increased mortality observed in FibγΔ⁵ mice.

The γ-chain AGDV motif is important for the binding of *S. aureus* to fibrin(ogen), but it is also a ligand for the platelet integrin αIIbβ₃, which plays a pivotal role in platelet aggregation [32,33]. Therefore, FibγΔ⁵ mice cannot effectively support fibrinogen-dependent platelet aggregation, but these animals retain normal platelet counts, plasma fibrinogen levels, clotting time, and fibrin crosslinking [27]. We found that platelet-depleted mice showed a modest, but significant, elevation in bacterial CFUs in the peritoneal lavage fluid compared to control mice. This finding is consistent with work from others showing that platelets contribute to host protection from *S. aureus* infection [34–36]. Additionally, clinical data has shown that patients that were thrombocytopenic at the onset of *S. aureus* bacteremia presented more commonly with severe sepsis that was accompanied by septic shock and renal failure [37]. Notably, the number of *S. aureus* CFUs present in the peritoneal cavity of FibγΔ⁵ mice (see Fig 3A) is higher than what is observed in both platelet-depleted mice (see Fig 5B) and mice infected with ClfA- *S. aureus* USA300 (see Fig 6A), suggesting that bacteria-fibrin(ogen) and platelet-fibrin(ogen) interactions may additively contribute to the host antimicrobial response to *S. aureus* peritonitis. The platelet contribution seems to be independent of thrombin-
PAR4-mediated activation, as mice lacking PAR4 on platelets showed similar bacterial CFU numbers in peritoneal lavage fluid, blood, and distant organs when compared to WT mice. This finding is consistent with previous studies showing that thrombin activity in the peritoneal cavity following S. aureus infection does not occur through host tissue factor, but through S. aureus coagulase function [19], and that S. aureus coagulase-prothrombin complexes (staphylothrombin) do not activate platelets directly [38].

WT mice infected with S. aureus deficient in ClfA showed a similar phenotype where there was significantly compromised bacterial clearance. However, whereas loss of the fibrinogen γ-chain AGDV motif also resulted in enhanced bacterial dissemination and accumulation of microbes in distant organs, loss of ClfA had only a minor impact on these aspects of the infection. We speculate that the increased CFUs of WT S. aureus USA300 observed in blood, heart, and lung of FibγAS mice are a result of the loss of interactions between the fibrinogen γ-chain AGDV motif and S. aureus factors other than ClfA. To this end, fibronectin-binding proteins Fnbp-A and Fnbp-B have been shown to bind this same region of fibrinogen [6]. It is possible that loss of fibrinogen binding with one or both of these S. aureus receptors, either alone or in combination with loss of ClfA-fibrin(ogen) binding, is the basis of increased CFUs in blood, heart, and lungs in FibγAS mice. Intriguingly, our findings are inconsistent with the concept that S. aureus produces ClfA to function as a potent virulence factor. One explanation is that ClfA plays a beneficial role for S. aureus in the context of certain types of infections or host microenvironments and that this outweighs the detrimental role it plays in peritonitis. For example, in a mouse model of bacteremia, intravenous infection with ClfA- S. aureus resulted in less host lethality than WT S. aureus [11]. S. aureus lacking ClfA have also been shown to be less virulent in mouse models of septic arthritis and endocarditis where the phenotypes have been attributed to loss of binding to fibrinogen deposited in inflamed joint tissue or damaged heart valves [7,39]. Further, ClfA has been shown to exacerbate S. aureus and formation of organ abscesses that shield the bacteria from host antimicrobial mechanisms and allow for proliferation [40]. Thus, the host compartment appears to be a significant determinant of bacteria virulence factor function.

Fibrin polymer formation is an integral component of the host antimicrobial mechanism as shown by studies performed using FibAEK mice. FibAEK mice possess a mutated form of fibrinogen that is not susceptible to proteolytic cleavage by thrombin or S. aureus coagulase-prothrombin complexes (i.e., staphylothrombin) [29]. FibAEK mice displayed significantly compromised bacterial clearance followed by enhanced dissemination from the peritoneal cavity following S. aureus infection (see Fig 7) [29]. Macrophages are key effector cells in the host antimicrobial response to S. aureus peritonitis [19,30,31], and engage fibrinogen via the leukocyte integrin receptor α4β2. The α4β2 binding motif is cryptic and is exposed by the conformational change that occurs following cleavage of fibrinogen to fibrin [41,42], consistent with the requirement of fibrin polymer formation for the antimicrobial response. Selectively eliminating fibrin-macrophage binding (using Fibγ390-396A mice) or macrophages themselves (using clodronate) resulted in a reduction in bacterial clearance from the lavage fluid and increased dissemination. Under these conditions, fibrinogen still accumulates in the peritoneal cavity and fibrin formation and S. aureus binding to fibrin(ogen) can still take place. Our findings suggest, fibrin matrix formation itself does not appear sufficient to retain the microbes in the peritoneal cavity. Whereas macrophage function is critical to the host antimicrobial response, the failure of bacterial killing observed in FibγAS mice was not linked to compromised phagocytosis as a significant increase in the number of bacteria internalized within host cells of the peritoneal cavity was observed. This increase in bacterial phagocytosis could serve as a ‘second hit’ to the host as it has been shown that once S. aureus are internalized within host cells, they suppress intracellular killing mechanisms and are shielded from other host
driven antimicrobial pathways which could result in a chronic infection [43–45]. Collectively, these data suggest the fibrin(ogen)-driven antimicrobial mechanism in the peritoneal cavity involves a tripartite complex of S. aureus-fibrin-macrophages (S4 Fig).

In this study, we show for the first time that the transglutaminase FXIII plays an important role in the host antimicrobial response against S. aureus peritonitis. F13a−/− mice show a modest but significant increase in S. aureus CFUs retrieved from the peritoneal cavity compared to WT mice following infection, suggesting a diminished host antimicrobial response. Deicke, et al. showed that FXIII enhances entrapment of Streptococcus pyogenes by crosslinking the bacteria to fibrin and that elimination of FXIII resulted in increased bacterial dissemination and poor survival in a mouse model of skin and soft tissue infection [46]. Other studies suggest FXIII can crosslink fibrin to the surface of bacteria, including S. aureus and E. coli, leading to their sequestration in clots [47]. Thus, a possible mechanism by which FXIII could enhance bacterial clearance is by preventing S. aureus from escaping fibrin matrices. Interestingly, while elimination of FXIII results in reduced clearance of S. aureus from the peritoneal cavity, we did not observe differences in bacterial CFUs in blood and distant organs when compared to WT mice. S. aureus produces a non-proteolytic activator of plasminogen called staphylokinase which allows the bacteria to escape entrapment by promoting fibrin degradation [6]. Fibrin that is not crosslinked by FXIII is more sensitive to degradation by plasmin [48], so it is possible that elimination of FXIII could allow S. aureus to more readily escape entrapment by proteolytically degrading fibrin matrices. Another possibility is that fibrin cross-linked by FXIII could function as a more efficient ligand for αMβ2 compared to non-cross-linked fibrin, thus driving a more potent macrophage-driven antimicrobial response. Currently, it is unknown if FXIII cross-linking has any effect of leukocyte binding via αMβ2. Further studies looking at binding of leukocytes to immobilized fibrinogen, fibrin and cross-linked fibrin would be necessary to understand these interactions.

The frequency of S. aureus as the causative agent in peritoneal infections is less pronounced than other Staphyloccocal species (e.g., S. epidermidis). Rather, bacteria lacking the virulence factors that engage host fibrinogen and other clotting system components integral to S. aureus virulence in bloodstream and soft tissue infection are prevalent sources of peritoneal infection [49]. In the peritoneal cavity, it is the host that utilizes clotting system proteins and many of the same S. aureus virulence factors to propagate a potent, acute, antimicrobial response. Current and previous work [29] has identified a deleterious effect of the FibγΔ5 and FibAEK mutations on host mortality following S. aureus peritonitis. However, additional studies are required to define the impact of eliminating the fibrin(ogen)-macrophage response, platelets, or fibrin crosslinking on host mortality; definitively linking specific fibrin-macrophage functions to the precise killing mechanism(s) for S. aureus, and potentially other microbes; and identify additional cells and antimicrobial molecules required for the antimicrobial response in the peritoneal cavity.

Materials and methods

Ethics statement

The University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (UNC-IACUC) approved all studies of mice performed under protocol number 19–204.

Mice. WT, FibγΔ5 [27], FibAEK [29], Fibγ390–396A [31], FXIII−/− [50] and Pf4-Cre+/PAR4fl/fl [51] mice were used in these studies. For each experiment, sex- and age-matched (i.e., both males and females of at 8–12 weeks of age) on a C57Bl/6J background were analyzed. Control mice were littermates derived from each colony analyzed.
Bacteria stains and growth conditions

WT and ClfA- *S. aureus* USA300 LAC were used in the studies conducted here. In addition, we used a complementation strain of ClfA- *S. aureus* USA300 LAC where the clfA gene and its 216bp upstream region were amplified from USA300 strain JE2 chromosomal DNA and inserted into plasmid pSK236 \[52\] by Gibson assembly, to yield plasmid pclfA. The plasmid insert was verified by sequencing prior to transformation into RN4220 and subsequently the USA300 ClfA- mutant strain. Stationary phase bacteria were grown in tryptic soy broth (TSB) (BD Difco) at 37˚C overnight, washed and re-suspended in phosphate-buffered solution (PBS), and diluted to an optical density (OD) at 600nm of 0.4, 1.0 or 6.0 based on the experiment. Exponential phase bacteria were grown in the same conditions overnight. The following morning, a small amount of the overnight culture was added to fresh TSB at a 1:50 ratio and incubated at 37˚C. Every 30 min, the OD$_{600}$ was measured until a value of 0.6 was reached. Then, cultures were washed and re-suspended in PBS, and diluted to an OD$_{600}$ of 0.4, 1.0 or 6.0 based on the experiment.

Fibrinogen purification

Fibrinogen was purified from citrate-plasma isolated from naïve Fibγ\(^{WT}\) and Fibγ\(^{Δ5}\) mice by ammonium sulfate precipitation. Briefly, whole blood is collected in 1:10 volume of 0.105 M citrate from inferior vena cava exsanguination and plasma isolated by centrifugation. Ammonium sulfate was added to plasma up to a concentration of 25% saturation to precipitate the fibrinogen, which was ultimately re-suspended in dialysis buffer (150mM NaCl, 20mM Hepes and 5mM ε-amino-n-caproic acid) and dialyzed overnight using the same buffer to remove any remaining ammonium sulfate.

Fibrinogen-*S. aureus* adhesion assay

NUNC 96-well plates (Thermo Fisher) were coated with 100μL of diluted purified mouse fibrinogen in buffer (15mM Na$_2$HCO$_3$, 35mM NaHCO$_3$, 3.2mM NaN$_3$) to concentrations ranging from 0.25–25μg/mL and incubated overnight at 4˚C. Following incubation, plates were washed three times with 100μL/well of Wash Buffer (150mM NaCl and 0.01% Tween20) and blocked with 100μL/well of 1% BSA, 0.05% Tween 20 solution in PBS. Plates were then incubated for 1hr at 37˚C and subsequently washed three times with 100μL/well of wash buffer. Afterwards, 100μL/well of *S. aureus* suspension was added to each well and subsequently incubated for 2hr at 37˚C. Bacterial suspensions contained either WT, ClfA- or ClfA-\(^{pclfa}\) *S. aureus* USA300 grown to stationary or exponential growth phase re-suspended at an OD$_{600}$ of 0.4. Plates were then washed 3 times with 100μL/well of wash buffer and fixed for 30 min with 25% formaldehyde solution. Once fixed, plates were washed once with 100μL/well of wash buffer and stained with 0.1% crystal violet for 30min. Stain was removed by washing plates 3 times with 100μL/well of wash buffer. Remaining stain was solubilized in 10% acetic acid and placed in a plate reader to quantify absorbance at 570nm.

Fibrinogen-*S. aureus* clumping assay

Purified mouse fibrinogen was diluted in PBS to suspensions ranging from 0.25–25μg/mL and 50μL/well was placed in 96-well tissue culture plates (BD Falcon). Stationary or exponential phase cultures of WT or ClfA-, ClfA-\(^{pclfa}\) *S. aureus* USA300 were prepared as a suspension at an OD$_{600}$ of 6.0, with 20μL/well of the bacterial suspension added to 96-well tissue culture plates. Plates were agitated using an orbital shaker for 5 min. Clumping was measured by reading light transmission at 570nm.
Mouse model of *S. aureus* peritonitis

Bacterial suspensions at a concentration of $\sim 1 \times 10^9$ CFUs/mL of stationary phase of WT or ClfA- USA300 *S. aureus* were prepared from overnight cultures and 1mL was administered by intraperitoneal injection to mice. After 1 or 4hr, mice were anesthetized with a cocktail of ketamine, xylazine and acepromazine followed by peritoneal lavage with 5mL of PBS, IVC blood draw and removal of the heart and lung. Serial dilutions of the lavage fluid, blood and homogenates of heart and lung were plated on Tryptic Soy Agar in duplicate and incubated overnight at 37˚C. Colony counts were performed for each sample and compared to the inoculum dose to determine bacterial clearance. For survival studies, 10 mice per group of Fibγ$^{\text{WT}}$ and Fibγ$^{\text{ΔS}}$, were administered an intraperitoneal injection of $\sim 5 \times 10^8$ CFUs of *S. aureus* and monitored for 7 days. Humane endpoints included the loss of up to 30% of the initial mouse body weight and/or reaching a moribund state.

Platelet and macrophage depletion

Platelets were depleted by intravenous injection of 2μg/g of anti-GPIb-α antibody (clone R300, Emfret Analytics) 3 hours prior to i.p. infection with WT *S. aureus* USA300. Following cell depletion, infection experiments were performed as described. Cell depletion was confirmed by flow cytometry analysis on blood collected 1hr after infection and stained with anti GPIX-AF488 antibody (Emfret Analytics). Macrophages were depleted using clodronate liposomes (Encapsula Nano Sciences) administered by intraperitoneal injection 24 hours prior to infection. Macrophage depletion was confirmed using FACS analysis in which cells from peritoneal lavage fluid were stained using BV605 Rat Anti-Mouse F4/80 (BD), and LIVE/DEAD Fixable Violet Cell Stain Kit (Thermo Fisher) and analyzed on the Attune flow cytometer. Total host cell counts within the lavage fluid were determined using a hemocytometer. Cytospin preparations of lavage fluid were stained with Kwik-Diff (Thermo Fisher Shandon) to determine differential host cell counts. Total cell counts were determined using a hemocytometer. Following cell depletion, infection experiments were performed as described.

Cytospin analysis

A 1:3 (100μL of lavage fluid + 200μL of PBS) dilution of peritoneal lavage fluid was prepared and 150μL of the solution was used for cytospin. Cytospin slides are let dry overnight and stained with Diff-Quick stain (methanol fixative, eosinophilic solution and basophilic solution). Stained slides were mounted using permount and imaged at 20 and 40x magnification. Quantification of peritoneal cells with internalized bacteria was performed by inspection of stained cytospin preparations under 20X magnification with at ~200 cells counted per sample. Representative images were captured at 40x.

Fibrinogen ELISA and Plasma Markers of Tissue Damage

Fibrinogen levels in peritoneal lavage fluid and citrated plasma were determined using Mouse Fibrinogen ELISA Kit (ICL). Tissue damage markers were measured in citrated plasma. Cardiac troponin I levels were determined by ELISA using a high-density mouse cardiac troponin I kit (Life Diagnostics, Inc.). Lactate dehydrogenase and creatine kinase were determined using a colorimetric activity assay (BioAssay Systems). Plasma alanine aminotransferase (ALT) levels were determined using an enzyme assay kit (Labs Biotechnology).
Supporting information

S1 Fig. *S. aureus* dissemination precedes overt tissue damage or increased intravascular coagulation. Circulating markers of tissue damage and coagulation activity were analyzed from mouse plasmas 4 hr after i.P. *S. aureus* infection. (A) Thrombin-anti-thrombin (TAT) complexes and (B) D-dimer were measured in circulation as markers of coagulation activation. Circulating levels of (C) lactate dehydrogenase (LDH) and (D) creatine kinase (CK) levels were analyzed as markers of muscle injury. (E) Alanine aminotransferase (ALT) as a marker of liver injury. (F) Circulating levels of cardiac troponin I was analyzed to assess damage to heart tissue. Data are presented as mean ± SEM and statistical significance was determined by 2-way ANOVA with Šidák’s multiple comparisons test.

(TIF)

S2 Fig. Mice infected with WT or ClfA- *S. aureus* show increased fibrinogen levels in lavage compared to uninfected mice. (A) Fibrinogen ELISA on lavage fluid from WT that received a sham injection or platelet depleting antibody and were infected with WT *S. aureus* USA300. (B) Fibrinogen ELISA on plasma from WT that received a sham injection or platelet-depleting GPI-α antibody and were infected with WT *S. aureus* USA300. (C) Fibrinogen ELISA on lavage fluid from WT mice that were uninfected or infected with WT or ClfA- USA300 *S. aureus*. (D) Fibrinogen ELISA on plasma from WT mice that were uninfected or infected with WT or ClfA- USA300 *S. aureus*. Data is presented as mean ± SEM and statistical significance was determined by One-way ANOVA with Tukey’s multiple comparisons test.

(TIF)

S3 Fig. Re-expression of ClfA in ClfA- *S. aureus* USA300 restores the infection profile observed with WT *S. aureus* USA300 in WT mice. Total live bacteria in (A) lavage, (B) blood, (C) heart and (D) Lung, and (E) Total host cells in WT mice 1 hour after i.p. infection with WT (1.07x10⁹ CFUs), ClfA- (1.19x10⁹ CFUs), or ClfA-* ΔclfA* (1.4x10⁹ CFUs) *S. aureus* USA300. Data is presented as mean ± SEM and statistical significance was determined by One-way ANOVA with Tukey’s multiple comparisons test. Dashed horizontal lines indicate the infection doses.

(TIF)

S4 Fig. Model of host antimicrobial response to *S. aureus* peritonitis. Following peritoneal *S. aureus* infection, fibrinogen enters the peritoneal cavity where it is converted to fibrin polymer by staphylothrombin and crosslinked by FXIIIa. Fibrin matrices bind both *S. aureus* and host macrophages via the γ-chain portion of the D-domain to drive a potent antimicrobial host defense response that kills the invading *S. aureus* and prevents dissemination. Platelets also contribute to the antimicrobial response but the mechanism is unknown.

(TIF)

Author Contributions

**Conceptualization:** Oscar Negrón, Joni Prasad, Jay L. Degen, Wolfgang Bergmeier, Magnus Höök, Matthew J. Flick.

**Data curation:** Oscar Negrón, Magnus Höök, Matthew J. Flick.

**Formal analysis:** Oscar Negrón, Matthew J. Flick.

**Funding acquisition:** Wolfgang Bergmeier, Magnus Höök, Matthew J. Flick.

**Investigation:** Oscar Negrón, Woosuk S. Hur, David S. Paul, Matthew J. Flick.
Methodology: Oscar Negro, David S. Paul, Sarah E. Rowe, Matthew J. Flick.

Project administration: Oscar Negro, Matthew J. Flick.

Resources: Oscar Negro, David S. Paul, Sarah E. Rowe, Silvio Antoniak, Brian P. Conlon, Wolfgang Bergmeier, Magnus Höök, Matthew J. Flick.

Supervision: Oscar Negro, Matthew J. Flick.

Validation: Oscar Negro, Matthew J. Flick.

Visualization: Oscar Negro, Matthew J. Flick.

Writing – original draft: Oscar Negro, Matthew J. Flick.

Writing – review & editing: Oscar Negro, Woosuk S. Hur, Joni Prasad, David S. Paul, Sarah E. Rowe, Jay L. Degen, Sara R. Abrahams, Silvio Antoniak, Brian P. Conlon, Wolfgang Bergmeier, Magnus Höök, Matthew J. Flick.

References

1. Brown AF, Leech JM, Rogers TR, McLoughlin RM. Staphylococcus aureus Colonization: Modulation of Host Immune Response and Impact on Human Vaccine Design. Front Immunol. 2014; 4:307. https://doi.org/10.3389/fimmu.2013.00507 PMID: 24409186

2. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998; 339(8):520–32. https://doi.org/10.1056/NEJM199808203390806 PMID: 9709046

3. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015; 28(3):603–61. https://doi.org/10.1128/CMR.00134-14 PMID: 26016486

4. Melzer M, Welch C. Thirty-day mortality in UK patients with community-onset and hospital-acquired methicillin-susceptible Staphylococcus aureus bacteraemia. J Hosp Infect. 2013; 84(2):143–50. https://doi.org/10.1016/j.jhin.2012.12.013 PMID: 23602415

5. Pottinger PS. Methicillin-resistant Staphylococcus aureus infections. Med Clin North Am. 2013; 97(4):601–19. x. https://doi.org/10.1016/j.mcn.2013.02.005 PMID: 23809716

6. Ko YP, Flick MJ. Fibrinogen Is at the Interface of Host Defense and Pathogen Virulence in Staphylococcus aureus Infection. Semin Thromb Hemost. 2016; 42(4):408–21. https://doi.org/10.1055/s-0036-1576639 PMID: 27056151

7. Moreillon P, Entenza JM, Francioli P, McDevitt D, Foster TJ, Francois P, et al. Role of Staphylococcus aureus coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect Immun. 1995; 63(12):4738–43. https://doi.org/10.1128/iai.63.12.4738-4743.1995 PMID: 7591130

8. Fei Y, Wang W, Kwiecinski J, Josefsson E, Pullerits R, Jonsson IM, et al. The combination of a tumor necrosis factor inhibitor and antibiotic alleviates staphylococcal arthritis and sepsis in mice. J Infect Dis. 2011; 204(3):348–57. https://doi.org/10.1093/infdis/jir266 PMID: 21742822

9. Que YA, Haefliger JA, Piroth L, Francois P, Widmer E, Entenza JM, et al. Fibrinogen and fibronectin binding cooperate for valve infection and invasion in Staphylococcus aureus experimental endocarditis. J Exp Med. 2005; 201(10):1627–35. https://doi.org/10.1084/jem.20050125 PMID: 15897276

10. McAdow M, Kim HK, Dedent AC, Hendrickx AP, Schneewind O, Missiajas DM. Preventing Staphylococcus aureus sepsis through the inhibition of its agglutination in blood. PLoS Pathog. 2011; 7(10):e1002907. https://doi.org/10.1371/journal.ppat.1002907 PMID: 22028651

11. Flick MJ, Du X, Prasad JM, Raghu H, Palumbo JS, Smeds E, et al. Genetic elimination of the binding motif on fibrinogen for the S. aureus virulence factor CiFA improves host survival in septicemia. Blood. 2013; 121(10):1783–94. https://doi.org/10.1182/blood-2012-09-453894 PMID: 23299312

12. Negron O, Flick MJ. Does fibrinogen serve the host or the microbe in Staphylococcus infection? Curr Opin Hematol. 2019; 26(5):343–8. https://doi.org/10.1097/MOH.0000000000000527 PMID: 31348048

13. Macrae FL, Duval C, Papareddy P, Baker SR, Yuldasheva N, Kearney KJ, et al. A fibrin biofilm covers blood clots and protects from microbial invasion. J Clin Invest. 2018; 128(8):3356–68. https://doi.org/10.1172/JCI98734 PMID: 29723163

14. Forsyth CB, Solovjov DA, Ugarova TP, Plow EF. Integrin alpha(M)beta2-mediated cell migration to fibrinogen and its recognition peptides. J Exp Med. 2001; 193(10):1123–33. https://doi.org/10.1084/jem.193.10.1123 PMID: 11369784
15. Languino LR, Plescia J, Duperray A, Brian AA, Plow EF, Geltosky JE, et al. Fibrinogen mediates leukocyte adhesion to vascular endothelium through an ICAM-1-dependent pathway. Cell. 1993; 73(7):1423–34. https://doi.org/10.1002/0008-8874(93)90367-y PMID: 8107042

16. Rubel C, Fernandez GC, Dran G, Bompadre MB, Istoriz MA, Palermo MS. Fibrinogen promotes neutrophil activation and delays apoptosis. J Immunol. 2001; 166(3):2002–10. https://doi.org/10.4049/jimmunol.166.3.2002 PMID: 11160249

17. Rubel C, Fernandez GC, Rosa FA, Gomez S, Bompadre MB, Coso OA, et al. Soluble fibrinogen modulates neutrophil functionality through the activation of an extracellular signal-regulated kinase-dependent pathway. J Immunol. 2002; 168(7):3527–35. https://doi.org/10.4049/jimmunol.168.7.3527 PMID: 11907115

18. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol. 2001; 167(5):2887–94. https://doi.org/10.4049/jimmunol.167.5.2887 PMID: 11509636

19. Prasad JM, Nepron O, Du X, Mullins ES, Palumbo JS, Gilbertie JM, et al. Host fibrinogen drives antimicrobial function in Staphylococcus aureus peritonitis through bacteria-mediated prothrombin activation. Proc Natl Acad Sci U S A. 2021; 118(1). https://doi.org/10.1073/pnas.2009837118 PMID: 33443167

20. Strong DD, Laudano AP, Hawiger J, Doolittle RF. Isolation, characterization, and synthesis of peptides from human fibrinogen that block the staphylococcal clumping reaction and construction of a synthetic clumping particle. Biochemistry. 1982; 21(6):1414–20. https://doi.org/10.1021/bi00535a048 PMID: 7074096

21. Ganesh VK, Rivera JJ, Smeds E, Ko YP, Bowden MG, Wann ER, et al. A structural model of the Staphylococcus aureus ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. PLoS Pathog. 2008; 4(11):e1000226. https://doi.org/10.1371/journal.ppat.1000226 PMID: 19043557

22. Wann ER, Gurusiddappa S, Hook M. The fibronectin-binding MSCRAMM FnbpA of Staphylococcus aureus is a bifunctional protein that also binds to fibrinogen. J Biol Chem. 2000; 275(18):13863–71. https://doi.org/10.1074/jbc.275.18.13863 PMID: 10789510

23. Ni Eidhin D, Perkins S, Francois P, Vaudaux P, Hook M, Foster TJ. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus. Mol Microbiol. 1998; 30(2):245–57. https://doi.org/10.1046/j.1365-2958.1998.00829.x PMID: 9791170

24. Wolz C, Pohllmann-Dietze P, Steinhuber A, Chien YT, Mann A, van Wamel W, et al. Agr-independent regulation of fibronectin-binding protein(s) by the regulatory locus sar in Staphylococcus aureus. Mol Microbiol. 2000; 36(1):230–43. https://doi.org/10.1046/j.1365-2958.2000.01853.x PMID: 10760180

25. Saravia-Otten P, Muller HP, Arvidson S. Transcription of Staphylococcus aureus fibronectin binding protein genes is negatively regulated by agr and an agr-independent mechanism. J Bacteriol. 1997; 179(17):5259–63. https://doi.org/10.1128/jb.179.17.5259-5263.1997 PMID: 9266974

26. Higgins J, Loughman A, van Kessel KP, van Strijp JA, Foster TJ. Clumping factor A of Staphylococcus aureus inhibits phagocytosis by human polymorphonuclear leucocytes. FEMS Microbiol Lett. 2006; 258(2):290–6. https://doi.org/10.1111/j.1574-6968.2006.00229.x PMID: 16640587

27. Holmback K, Danton MJ, Suh TT, Daugherty CC, Degen JL. Impaired platelet aggregation and sustained bleeding in mice lacking the fibrinogen motif bound by integrin alpha IIb beta 3. EMBO J. 1996; 15(21):5760–71. PMID: 8918453

28. Mullins ES, Kombirck KW, Talmage KE, Shaw MA, Witte DP, Ullman JM, et al. Genetic elimination of the fibrinogen motif bound by integrin alpha IIb beta 3 in mice results in spontaneous hemorrhagic events in both heart and brain. Blood. 2009; 113(3):696–704. https://doi.org/10.1182/blood-2008-07-169003 PMID: 18927430

29. Prasad JM, Gorkun OV, Raghu H, Thornton S, Mullins ES, Palumbo JS, et al. Mice expressing a mutant form of fibrinogen that cannot support fibrin formation exhibit compromised antimicrobial host defense. Blood. 2015; 126(17):2047–58. https://doi.org/10.1182/blood-2015-04-639849 PMID: 26228483

30. Brown AF, Murphy AG, Lalor SJ, Leech JM, O’Keeffe KM, Mac Aogain M, et al. Memory Th1 Cells Are Protective in Invasive Staphylococcus aureus Infection. PLoS Pathog. 2015; 11(11):e1005226. https://doi.org/10.1371/journal.ppat.1005226 PMID: 26539822

31. Flick MJ, Du X, Witte DP, Jurkoskova M, Solovev DA, Busuttil SJ, et al. Leukocyte engagement of fibrinogen (ogen) via the integrin receptor alphaMbeta2/Mac-1 is critical for host inflammatory response in vivo. J Clin Invest. 2004; 113(11):1596–606. https://doi.org/10.1172/JCI20741 PMID: 15173886

32. Caen JP, Rosa JP. Platelet-vessel wall interaction: from the bedside to molecules. Thromb Haemost. 1995; 74(1):18–24. PMID: 8578453

33. Nurden AT, Nurden P. Congenital platelet disorders and understanding of platelet function. Br J Haematol. 2014; 165(2):165–78. https://doi.org/10.1111/bjh.12662 PMID: 24286193
34. Wuescher LM, Takashima A, Worth RG. A novel conditional platelet depletion mouse model reveals the importance of platelets in protection against Staphylococcus aureus bacteremia. J Thromb Haemost. 2015; 13(2):303–13. https://doi.org/10.1111/jth.12795 PMID: 25418277

35. Yeaman MR, Norman DC, Bayer AS. Platelet microbicidal protein enhances antibiotic-induced killing of and postantibiotic effect in Staphylococcus aureus. Antimicrob Agents Chemother. 1992; 36(8):1665–70. https://doi.org/10.1128/AAC.36.8.1665 PMID: 1416849

36. Trier DA, Gank KD, Kuperwasser D, Yount NY, French WJ, Michelson AD, et al. Platelet antistaphylococcal responses occur through P2X1 and P2Y12 receptor-induced activation and kinocidin release. Infect Immun. 2008; 76(11):5706–13. https://doi.org/10.1128/IAI.00935-08 PMID: 18824536

37. Gafter-Gril A, Mansur N, Bivas A, Zemer-Wassercug N, Bishara J, Leibovici L, et al. Thromboctypenia in Staphylococcus aureus bacteremia: risk factors and prognostic importance. Mayo Clin Proc. 2011; 86(5):389–96. https://doi.org/10.4065/mcp.2010.0705 PMID: 21538182

38. Vanassche T, Kauskot A, Verhaegen J, Peetermans WE, van Ryn J, Schneewind O, et al. Fibrin formation by staphylothrombin facilitates Staphylococcus aureus-induced platelet aggregation. Thromb Haemost. 2012; 107(6):1107–21. https://doi.org/10.1160/TH11-12-0891 PMID: 22437005

39. Josefsson E, Hartford O, O’Brien P, Patti JM, Foster T. Protection against experimental Staphylococcus aureus arthritis by vaccination with clumping factor A, a novel virulence determinant. J Infect Dis. 2001; 184(12):1572–80. https://doi.org/10.1086/324430 PMID: 11740737

40. Cheng AG, Kim HK, Burts ML, Krauss T, Berke G, Li M, Smith AG, Lathe R, et al. Targeted inactivation of the mouse locus encoding coagulation factor XIII: hemostatic abnormalities in mutant mice and characterization of the coagulation deficit. Thromb Haemost. 2002; 88(5):389–96. https://doi.org/10.1160/10.1111/j.1354-7339.2002.tb08616.x PMID: 12390300

41. Loike JD, Silverstein R, Wright SD, Weitz JI, Huang AJ, Silverstein SC. The role of protected extracellular compartments in interactions between leukocytes, and platelets, and fibrin/fibrinogen matrices. Ann N Y Acad Sci. 1992; 667:163–72. https://doi.org/10.1111/j.1749-6632.1992.tb1608X PMID: 1309302

42. Lishko VK, Kudryk B, Yakunenko VP, Yee VC, Ugarova TP. Regulated unmasking of the cryptic binding site for integrin alpha M beta 2 in the gamma C-domain of fibrinogen. Biochemistry. 2002; 41(43):12942–51. https://doi.org/10.1021/bi026324c PMID: 12390050

43. Koziel J, Maciag-Gudowska A, Mikolajczyk T, Bzowska M, Sturdevant DE, Whitney AR, et al. Phagocytosis of Staphylococcus aureus by macrophages exerts cytoprotective effects manifested by the upregulation of antiapoptotic factors. PLoS One. 2009; 4(4):e5210. https://doi.org/10.1371/journal.pone.0005210 PMID: 19381294

44. Joch SK, Surewaard BG, Hossain M, Hossaert M, Peiseler M, Deppermann C, Deng J, et al. Peritoneal GATA6+ macrophages function as a portal for Staphylococcus aureus dissemination. J Clin Invest. 2019; 129(11):4643–56. https://doi.org/10.1172/JCI127286 PMID: 31545300

45. Neupane R, Bhatt N, Poudyal A, Sharma A, Methicillin-Resistant Staphylococcus Aureus Nasal Carriage among Laboratory Technical Staff of Tertiary Hospital in Eastern Nepal. Kathmandu Univ Med J (KUMJ). 2020; 18(69):3–8. PMID: 33582679

46. Deicke C, Chakrakodi B, Pilis MC, Dicknete G, Johansson L, Medina E, et al. Local activation of coagulation factor XIII reduces systemic complications and improves the survival of mice after Streptococcus pyogenes M1 skin infection. J Med Microbiol. 2016; 306(7):572–9. https://doi.org/10.1096/fmm.09-13546 PMID: 27338836

47. Wang Z, Wilhemsson C, Hyrs P, Loof TG, Dobes P, Klupp M, et al. Pathogen entrapment by transglutaminase—a conserved early innate immune mechanism. PLoS Pathog. 2010; 6(2):e1000763. https://doi.org/10.1371/journal.ppat.1000763

48. Rijken DC, Abdul S, Malfliet JJ, Leebeek FW, Uitte De Willige S. Compaction of fibrin clots reveals the antifibrinolytic effect of factor XIII: reply. J Thromb Haemost. 2017; 15(1):205–6. https://doi.org/10.1111/jth.13544 PMID: 27748985

49. Camargo CH, Cunha Mde L, Caramori JC, Mondelli AL, Montelli AC, Barretti P. Peritoneal dialysis-related peritonitis due to coagulase-negative Staphylococcus: a review of 115 cases in a Brazilian center. Clin J Am Soc Nephrol. 2014; 9(6):1074–81. https://doi.org/10.2215/CJN.09280913 PMID: 24677560

50. Lauer P, Metzner HJ, Zettlmeissl G, Li M, Smith AG, Lathe R, et al. Targeted inactivation of the mouse locus encoding coagulation factor XIII-A: hemorrhagic abnormalities in mutant mice and characterization of the coagulation deficit. Thromb Haemost. 2002; 88(6):967–74. PMID: 12529747

51. Lee RH, Kawano T, Grover SP, Bharath V, Martinez D, Cowley DO, et al. Genetic deletion of platelet PAR4 results in reduced thrombosis and impaired hemostatic plug stability. J Thromb Haemost. 2021. https://doi.org/10.1111/jth.15569 PMID: 34689407

52. Chien Y, Mann A, Projan SJ, Cheung AL. SarA, a global regulator of virulence determinants in Staphylococcus aureus, binds to a conserved motif essential for sar-dependent gene regulation. J Biol Chem. 1999; 274(52):37169–76. https://doi.org/10.1074/jbc.274.52.37169 PMID: 10601279