Contribution of *Staphylococcus aureus* Coagulases and Clumping Factor A to Abscess Formation in a Rabbit Model of Skin and Soft Tissue Infection

Natalia Malachowa¹, Scott D. Kobayashi¹, Adeline R. Porter¹, Kevin R. Braughton¹, Dana P. Scott², Donald J. Gardner², Dominique M. Missiakas³, Olaf Schneewind³, Frank R. DeLeo¹*

¹ Laboratory of Bacteriology, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, United States of America, ² Rocky Mountain Veterinary Branch, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, United States of America, ³ Department of Microbiology, University of Chicago, Chicago, Illinois, United States of America

* fdeleo@niaid.nih.gov

Abstract

*Staphylococcus aureus* produces numerous factors that facilitate survival in the human host. *S. aureus* coagulase (Coa) and von Willebrand factor-binding protein (vWbp) are known to clot plasma through activation of prothrombin and conversion of fibrinogen to fibrin. In addition, *S. aureus* clumping factor A (ClfA) binds fibrinogen and contributes to platelet aggregation via a fibrinogen- or complement-dependent mechanism. Here, we evaluated the contribution of Coa, vWbp and ClfA to *S. aureus* pathogenesis in a rabbit model of skin and soft tissue infection. Compared to skin abscesses caused by the Newman wild-type strain, those caused by isogenic *coa*, *vwb*, or *clfA* deletion strains, or a strain deficient in *coa* and *vwb*, were significantly smaller following subcutaneous inoculation in rabbits. Unexpectedly, we found that fibrin deposition and abscess capsule formation appear to be independent of *S. aureus* coagulase activity in the rabbit infection model. Similarities notwithstanding, *S. aureus* strains deficient in *coa* and *vwb* elicited reduced levels of several proinflammatory molecules in human blood *in vitro*. Although a specific mechanism remains to be determined, we conclude that *S. aureus* Coa, vWbp and ClfA contribute to abscess formation in rabbits.

Introduction

*Staphylococcus aureus* remains one of the most prominent human bacterial pathogens worldwide [1, 2]. These Gram-positive cocci cause a wide clinical spectrum of disease and/or syndromes, including endocarditis, bacteremia, pneumonia, toxic shock syndrome, osteomyelitis, and skin and soft tissue infections (SSTIs) [3–5]. The remarkable success of *S. aureus* as a human pathogen is facilitated by its vast arsenal of virulence factors and an ability to acquire antibiotic resistance readily [5, 6].
Coagulase (Coa) is one of the earliest described virulence factors of S. aureus [7], and is routinely used as a diagnostic tool to differentiate between two major species of Staphylococcus, i.e., coagulase-positive (S. aureus) and coagulase-negative (e.g., S. epidermidis) organisms. Recently, a second S. aureus coagulase was discovered and named von Willebrand factor-binding protein (vWbp) [8]. Coa and vWbp display sequence and structure homology, particularly at the N-terminus [9]. Both proteins insert N-terminal residues into the prothrombin zymogen cleft, which triggers non-proteolytic activation by conformational transformation and formation of a staphylothrombin complex [10, 11]. The C-terminal domain (substrate recognition domain) of coagulase binds fibrinogen, which is transformed into fibrin and subsequently forms a fibrin clot.

Fibrin deposition is a process critical to abscess formation and thereby contributes to host defense against invading S. aureus [12]. The S. aureus coagulases have been linked previously to abscess development in murine systemic [13] and subcutaneous models of infection [14]. Clumping factor A (ClfA), although not a coagulase, is a fibrinogen binding protein that can promote fibrinogen-dependent platelet aggregation and adherence of S. aureus to fibrin [15, 16]. Similar to the coagulases, a role for ClfA in S. aureus abscess formation has been demonstrated in murine models of S. aureus virulence [17–19].

Rabbit models of S. aureus infection were used historically to investigate virulence and host-pathogen interactions, but were replaced largely by mouse infection models. Although neither mouse nor rabbit innate immune systems faithfully recapitulate that of humans, there are characteristics of the rabbit innate immune system—especially those of granulocytes—that seem more closely aligned with those of humans by comparison. A role for coagulases and ClfA has not been reported in a rabbit model of S. aureus SSTI. To that end, we evaluated the role of S. aureus coa, vwb, and clfA in a rabbit skin abscess model.

Materials and Methods

Ethics statement

All animal studies and procedures were approved by the Animal Care and Use Committee at Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases (NIAID) under protocols 2011–92 and 2012–027, and conformed to the guidelines of the National Institutes of Health (NIH).

Human venous blood was obtained from healthy donors according to a protocol approved by the Institutional Review Board for Human Subjects, NIAID, NIH. Studies were conducted according to the policies provided in the Declaration of Helsinki, and each volunteer provided written informed consent prior to participation in the study.

Bacterial strains and growth conditions

S. aureus Newman wild-type and isogenic coa (Δcoa), vwb (Δvwb), and coa/vwb (Δcoa/Δvwb) deletion strains, and a clfA transposon mutant strain (ΔclfA), were described previously [13, 20, 21]. Briefly, the pKOR1 allelic replacement system was used to create the S. aureus Δcoa, Δvwb and Δcoa/Δvwb deletion strains [13, 22], and the mariner-based bursa aurealis transposon system was used to construct the ΔclfA strain [21]. All S. aureus strains used for these studies have been phenotypically evaluated for their ability to clot whole blood [13]. We used S. aureus strains Newman, Δcoa, Δvwb, and Δcoa/Δvwb since they were readily available and used previously in murine models of S. aureus abscess formation [13, 21]. Bacteria were cultured in trypticase soy broth (TSB; Difco, Detroit, MI) at 37°C with constant shaking at 225 rpm. Overnight cultures were diluted 1:200 into fresh TSB and grown to early stationary (OD₆₀₀ ~ 2.0) growth phase prior to use in assays.

PLOS ONE | DOI:10.1371/journal.pone.0158293 June 23, 2016 2/14
Rabbit skin and soft tissue infection model

Animal experiments were performed as described [23]. Briefly, bacteria were cultured to early stationary phase of growth and then pelleted by centrifugation. Cells were washed twice with Dulbecco’s phosphate-buffered saline (DPBS; Sigma-Aldrich, St. Louis, MO) and suspended in sterile DPBS at $5 \times 10^9$ colony-forming units (CFU)/ml. The *S. aureus* dose used in this study was determined empirically in rabbits and results in reproducible abscesses that are easily evaluated by gross morphology [23]. *S. aureus* inocula were verified by enumeration of CFUs on trypticase soy agar plates. Five rabbits (NZW, strain Cr1c:KBL; Western Oregon Rabbit Company, Philomath, OR) were used for each group and each group was infected with a different *S. aureus* strain. Rabbits were anesthetized and subsequently inoculated with 100 μl of bacterial suspension into the left and right flank (5 rabbits for each strain and thus 10 abscesses per strain), and 100 μl of DPBS was injected into lower right flank for use as a sham infection control. Animals were monitored daily and allowed food and water ad libitum. *S. aureus* inflammatory lesions were measured daily for 14 days with a caliper as described previously [23]. Experiments were repeated twice using an additional set of two animals per strain to assess *S. aureus* abscess CFUs on day 2 post infection, and one animal per strain/day was used for histopathology analysis. Animals were humanely euthanized prior to tissue excision in accordance with protocol approved by the Institutional Animal Care and Use Committee.

Histopathology analysis

Abscesses with margins of surrounding tissue were excised and fixed in 10% neutral-buffered formalin for at least 48 hours and processed as described [24]. Tissues sections were stained with hematoxylin-eosin, Masson’s trichrome stain for capsule or Mallory’s phosphotungstic acid-hematoxylin for fibrin visualization [25]. Images of tissue sections were captured using an Olympus model BX-51 microscope and Olympus cellSens Dimension 1.13 software (Olympus, Center Valley, PA).

Quantitative analysis of molecules produced in human whole blood in response to *S. aureus*

Bacteria at mid-logarithmic growth phase were pelleted by centrifugation, washed twice with Dulbecco’s phosphate-buffered saline (DPBS; Gibco/Life Technologies, Grand Island, NY) and suspended in sterile RPMI 1640 medium buffered with 10 mM HEPES (RPMI/H; Invitrogen/Life Technologies, Grand Island, NY). Bacteria were added to heparinized human blood at a final concentration of $1 \times 10^6$ CFU/ml. A 1-ml sample of blood culture was taken immediately to serve as a time zero control and the remaining samples were incubated for 2 h at 37°C with gentle rotation. The blood-bacteria mixture was centrifuged at 1300 × g for 10 min at 25°C to collect plasma for analysis of inflammation molecules. Samples were stored at -80°C until shipped for analysis (Multi-Analyte Profiling (MAP) technology platform (HumanMap® v.2.0; Myriad RBM, Inc., Austin, TX). Data sets were analyzed using a one-way ANOVA and Tukey’s post-test. The complete results of the HumanMap analysis are provided in S1 Table.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA). Data for abscess size were evaluated with a one-way ANOVA and Dunnett’s post-test to correct for multiple comparisons.
Results

SSTIs are among the most common manifestations of *S. aureus* disease. We previously developed a rabbit SSTI model to assess the relative contribution of USA300 virulence determinants to CA-MRSA pathogenesis [23]. Inasmuch as the *S. aureus* coagulases and ClfA contribute to fibrin deposition and are linked to abscess formation in murine infection models, we compared the ability of *S. aureus* Newman wild-type, Δcoa, Δvwb, Δcoa/Δvwb, and ΔclfA strains to cause abscesses in our rabbit SSTI model. Rabbits were infected by subcutaneous inoculation of *S. aureus* strains and abscess development (lesion size and assessment of gross morphology) was monitored daily for 14 days. All *S. aureus* strains tested caused formation of typical skin abscesses, as determined by gross morphology [23]. However, there were strain-dependent differences in abscess size (Fig 1A and 1B and S1 Fig). For example, abscesses caused by the Newman wild-type strain were significantly larger than those caused by Δcoa, Δvwb, or Δcoa/Δvwb strains on days 1, 2, 3, and 5 post-infection (*P < 0.05, Fig 1A*). Although abscesses caused by the ΔclfA strain were smaller than those caused by the wild-type strain (*P < 0.05 on day 5*), the difference was less pronounced compared to that of the coagulase negative strains (Fig 1A).

To determine if abscess size is associated with (or linked directly to) bacterial burden, we performed a second set of experiments to evaluate *S. aureus* CFUs in rabbit abscesses on day 2 following subcutaneous inoculation with each strain (Fig 1C and 1D). Unexpectedly, we found that CFUs per abscess were similar in all strains tested, indicating that the decreased abscess size (relative to wild-type) for the mutant strains was not due to a decrease in viability or more rapid bacterial clearance in this model. These findings contrast with those reported previously for ΔclfA [26, 27], Δvwb and Δcoa strains [13, 28] in murine abscess models of *S. aureus* infection. It is possible differences in animal species (mouse versus rabbit) and infection models employed account for the differences in results with bacterial burden.

Inasmuch as *S. aureus* coagulases and ClfA are involved in fibrin deposition, and since there were no apparent differences in bacterial numbers within abscesses, we next examined histological sections to ascertain differences in abscess fine structure (Fig 2 and Table 1). Abscesses were surgically excised on days 2, 6, and 10 following s.c. inoculation with *S. aureus*, and histopathology sections were processed and scored (Table 1). We found that all *S. aureus* strains tested caused formation of structurally discrete abscesses surrounded by a fully developed fibrous capsule by day 10, and that there were limited differences revealed by abscess histopathology—regardless of the *S. aureus* strain used for infection (Fig 2 and Table 1). Moreover, our data indicate that deposition of fibrin during abscess formation was mostly independent of Coa, vWbp and ClfA activity. Nonetheless, abscesses induced by ΔclfA had relatively weak fibrin deposition that was apparent only in abscesses excised on Day 2 and 10 (Fig 3). These data correspond with those in the mouse SSTI model, where fibrin deposition was apparent in abscesses induced by *S. aureus* when coagulase activity was blocked by dabigatran [14].

Abscesses caused by the Newman wild-type strain scored categorically highest on each day based on histopathology features, with few exceptions (Table 1). One notable distinction was that abscesses from rabbits infected with the ΔclfA strain had no evidence of thrombosis and weak vascular necrosis in the majority of sections analyzed. Although present at the earlier time points, vascular necrosis was also limited in tissue samples from abscess caused by coagulase deficient strains (Fig 4 and Table 1).

The acute inflammatory response associated with *S. aureus* SSTI is triggered at least in part by production of pro-inflammatory signaling molecules and rapid recruitment of immune cells. To gain insight into the role of *S. aureus* coagulases and ClfA in the induction of host inflammation, we utilized a multi-analyte profiling approach to measure immune molecule production in human blood in response to *S. aureus* Newman wild-type, ΔclfA, Δcoa, Δvwb,
Fig 1. Contribution of S. aureus coa, vwb and clfA to formation of rabbit abscesses. (A) Average abscess volume for rabbits infected subcutaneously with S. aureus Newman wild-type (WT) or isogenic mutant strains as indicated. The volume of 10 abscesses per bacterial strain was measured daily following inoculation. (B) Individual abscesses plotted for selected days are depicted in panel A. (C) A separate set of 2 animals (4 abscesses/strain) was used to determine S. aureus CFU per abscess on day 2 post-infection and (D) the volume of rabbit abscesses. Each symbol represents a data point obtained from a single abscess. $P$ values were calculated using a one-way ANOVA and Dunnett’s post-test.

doi:10.1371/journal.pone.0158293.g001
We performed the protein profiling experiments using human blood because there is paucity of reagents available to analyze a comprehensive panel of rabbit immune mediators. We also demonstrated previously that human and rabbit blood incubated with *S. aureus* have similar proinflammatory cytokine gene transcription profiles [29, 30]. As anticipated, several proinflammatory molecules were upregulated in blood samples incubated with *S. aureus*.

**Fig 2.** Histopathology of rabbit skin abscess caused by *S. aureus*. Histopathology sections represent skin abscesses caused by *S. aureus* Newman WT (A, B), ΔclfA (C, D) or Δcoa/Δvwb (E, F) strains on day 10 post-infection. Abscess sections were stained with standard Masson’s trichrome stain to enhance fine structure detail of muscle tissues, collagen fibers and fibrin. (A, C and E) original magnification is 20x. (B, D, and F) 200x magnification of selected area (black rectangle) depicted in panels A, C or E.

doi:10.1371/journal.pone.0158293.g002
### Table 1. Contribution of coa, vwb and clfA to structure and development of the abscess.

| Strain                  | Days post-infection | WT   | ΔclfA | Δcoa | Δvwb | Δcoa/Δvwb | PBS ctrl |
|-------------------------|---------------------|------|-------|------|------|-----------|----------|
| Capsule granulation tissue | d2/6/10             | d2   | d6    | d10  | d2   | d6        | d10      |
| Fibrous capsule         | 0/2                 | 2/2  | 2/2   | 0/2  | 2/2  | 0/2       | 2/2      |
| Epithelialization       | 0/2                 | 2/2  | 1/2   | 0/2  | 2/2  | 2/2       | 0/2      |
| Vasculitis/vascular necrosis | 2/2                | 2/2  | 2/2   | 1/2  | 0/2  | 2/2       | 2/2      |
| Thrombosis              | 2/2                 | 2/2  | 1/2   | 0/2  | 2/2  | 2/2       | 2/2      |
| Coagulative necrosis    | 2/2                 | 2/2  | 1/2   | 0/2  | 2/2  | 1/2       | 2/2      |
| Extracellular bacteria  | 2/2                 | 2/2  | 2/2   | 2/2  | 2/2  | 2/2       | 2/2      |
| Intracellular bacteria  | 2/2                 | 2/2  | 2/2   | 2/2  | 2/2  | 2/2       | 2/2      |
| Compiled score          | 10/16               | 14/16| 13/16 | 5/16 | 8/16 | 11/16     | 7/16     |

Abscesses were scored based on the presence (1) or absence (0) of a chosen feature based on 6 histopathology sections of two abscesses (3 each) for each bacterial strain. 0/2 = present in neither abscess; 1/2 = present in 1 of 2 abscesses; 2/2 = present in both abscesses.

doi:10.1371/journal.pone.0158293.t001
for 2 h compared to control blood lacking bacteria, including interleukin (IL)-8, myeloperoxidase (MPO), tumor necrosis factor (TNFα), and vascular endothelial growth factor (VEGF) (Fig 5 and S1 Table). There were also notable differences in levels of proinflammatory molecules elicited by *S. aureus* mutant and wild-type strains tested. For example, there was reduced expression of key proinflammatory mediators (IL-1β, MPO, PAI-1 and ENA-78) in human blood incubated with the *S. aureus* Δcoa/Δvwb strain compared to the wild-type Newman strain (Fig 5). If this phenomenon can be extended to host responses in tissues, it could provide...
Fig 4. Vasculitis caused by *S. aureus* Newman WT. Histopathology sections of rabbit abscesses depicting vascular necrosis caused by *S. aureus* Newman WT (A), and an intact artery within a Δcoa/Δvwb induced abscess (B) or PBS control (C) on day 10 post infection. Original magnification is 100×.

doi:10.1371/journal.pone.0158293.g004
Fig 5. *S. aureus* Newman causes increased production of proinflammatory molecules in human whole blood. *S. aureus* was cultured in human heparinized blood for 2 h. Accumulation of proinflammatory molecules in plasma was evaluated by quantitative, multiplexed immunoassays (HumanMAP v2.0; Myriad RBM) as described in Materials and Methods section. Data represents average of 3 donors with one-way ANOVA and Tukey’s post-test used to determine statistical significance. *P* < 0.05 for the selected pairs; # *p* < 0.05 compared to uninfected blood sample (ctrl).

doi:10.1371/journal.pone.0158293.g005
an explanation in part for diminished pathology caused by the mutant strains in the rabbit SSTI model of infection, albeit this hypothesis requires further investigation.

**Discussion**

The rabbit is historically the classical animal model for studying *S. aureus* pathogenesis [31, 32] and has been used to model a diversity of diseases and syndromes such as endocarditis, pneumonia, sepsis, and toxemia [33–37]. We recently developed a rabbit model of skin and soft tissue infection [23] to study the contribution of *S. aureus* leukotoxins to abscess formation. *In vitro* studies indicate that susceptibility of rabbit cells to several *S. aureus* secreted leukotoxins and hemolysins approximates that of human cells more closely than those of murine origin [38, 39]. For example, mouse leukocytes are less susceptible (7–10 fold) to the cytolytic effects of PVL compared with human or rabbit leukocytes, and purified PVL has been tested directly in a rabbit skin infection model [40].

Inasmuch as *S. aureus* Coa, vWbp and ClfA are linked previously to abscess formation following murine systemic infection, we employed the rabbit skin and soft tissue infection model to assess the role of coagulase in development of subcutaneous abscesses. Our data indicate that all three of these molecules contribute to the formation of *S. aureus* abscesses in the experimental rabbit infection model. However, we found that the direct contribution of *S. aureus* Coa and vWbp to capsule formation and fibrin deposition was limited (Figs 2 and 3) compared to that reported for the *S. aureus* murine kidney model [13]. There are a couple of potential explanations for differences observed between the infection models. First, there are significant differences in the host proinflammatory response to invading pathogens between mice and rabbits, and as an example, IL-8 is a factor critical for neutrophil recruitment in humans and rabbits but is absent in mice [41, 42]. While it is evident that proinflammatory mediators play a critical role in formation of *S. aureus* abscesses [43–46], it is unlikely that species-specific production of proinflammatory molecules contribute to the differences reported for the role of coagulases on abscess structure between the models. On the other hand, it is possible that the role of *S. aureus* coagulases on abscess structure differs depending on the anatomical location of the abscess, rather than the animal species tested. Renal abscesses form as a result of systemic infection, during which disseminated bacteria within host blood accumulate in blood filtration organs such as the kidney or liver. *S. aureus* commonly accumulates in the renal arcuate arteries and causes infarcts [47, 48]. The combination of bacteria and tissue damage elicits neutrophil and other immune cell infiltration, and facilitates formation of a mature abscess. By contrast, invading *S. aureus* are recognized early during SSTI by local keratinocytes and resident skin monocytes, which initiate cytokine signaling to promote immune cell recruitment [49]. This triggers influx of neutrophils to the infection site to initiate the process of abscess formation [12, 50]. The influx of neutrophils also contributes to increased vascular permeability at the site of inflammation [51, 52]. Since coagulases and clumping factor A function primarily through binding or modifying fibrinogen—one of the most abundant plasma glycoproteins [53]—it is possible that limited access to fibrinogen in subcutaneous tissue reduces the role of coagulases and/or ClfA in formation of the SSTI abscess compared to the kidney. Indeed, consistent with our findings in rabbits, a *S. aureus* strain deficient for coa and vwb formed smaller subcutaneous abscesses in murine SSTI, and inhibition of the staphylothrombin complex by dabigatran treatment did not prevent deposition of fibrin and fibrinogen within the *S. aureus* wild-type abscess capsule [14]. However, in that study, abscess structure was not assessed directly by histopathology following infection with the *S. aureus* Δcoa/Δvwb deletion strain. Nevertheless, more work is needed to determine if there are variations in organ-specific immune response and/or bacterial response that may influence abscess development.
Collectively, the data obtained from our rabbit infection model confirm previous findings that Coa, vWbp and ClfA are involved in the pathogenesis of *S. aureus* SSTI and contribute to the host proinflammatory response to infection.

**Supporting Information**

S1 Fig. Rabbit abscess volume following infection with *S. aureus* wild type and Δcoa, Δvwb, ΔclfA, and Δcoa/Δvwb isogenic deletion strains. Scatter plot of abscess volumes from data shown in Fig 1A. Rabbits were infected subcutaneously with *S. aureus* Newman wild-type (WT) or isogenic mutant strains. The volume of 10 abscesses per bacterial strain was measured for 14 days following inoculation. Each symbol represents a data point obtained from a single abscess. (TIF)

S1 Table. Production of proinflammatory molecules in human whole blood after incubation with *S. aureus* Newman strain and its isogenic mutants. *S. aureus* was cultured in human heparinized blood up to 2 h. Accumulation of proinflammatory molecules in plasma was evaluated by quantitative, multiplexed immunoassays (HumanMAP v2.0; Myriad RBM) as described in Materials and Methods section. Data represents average of 3 donors ±SEM. (DOCX)

**Author Contributions**

Conceived and designed the experiments: NM SDK DMM OS FRD. Performed the experiments: NM SDK ARP KRB DPS DJG. Analyzed the data: NM SDK DPS DMM OS FRD. Contributed reagents/materials/analysis tools: DMM OS. Wrote the paper: NM SDK FRD.

**References**

1. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med. 2006; 355: 666–74. PMID:16914702

2. Boucher H, Miller LG, Razonable RR. Serious infections caused by methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 2010; 51: S183–S97. doi:10.1086/653519 PMID: 20731576

3. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jernigan JA, et al. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N Engl J Med. 2005; 352: 1436–44. PMID: 15814879

4. Landrum ML, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, et al. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005–2010. JAMA. 2012; 308: 50–9. doi: 10.1001/jama.2012.7139 PMID: 22760291

5. Lowy FD. *Staphylococcus aureus* Infections. N Engl J Med. 1998; 339: 520–32. PMID: 9709046

6. Kobayashi SD, Musser JM, DeLeo FR. Genomic analysis of the emergence of vancomycin-resistant *Staphylococcus aureus*. MBio. 2012; 3: e00170–12. doi: 10.1128/mBio.00170-12 PMID: 22738541

7. Loeb L. The influence of certain bacteria on the coagulation of the blood. J Med Res. 1903; 10: 407–19. PMID: 19971581

8. Bjerketorp J, Nilsson M, Ljungh Å, Flock J-I, Jacobsson K, Frykberg L. A novel von Willebrand factor binding protein expressed by *Staphylococcus aureus*. Microbiology. 2002; 148: 2037–44. PMID: 12101292

9. McAdow M, DeDent AC, Emolo C, Cheng AG, Kreiswirth BN, Missiakas DM, et al. Coagulases as determinants of protective immune responses against *Staphylococcus aureus*. Infect Immun. 2012; 80: 3389–98. doi: 10.1128/IAI.00562-12 PMID: 22825443

10. Friedrich R, Panizzi P, Fuentes-Prior P, Richter K, Verhamme I, Anderson PJ, et al. Staphylocoagulase is a prototype for the mechanism of cofactor-induced zymogen activation. Nature. 2003; 425: 535–9. PMID: 14523451
16. McDevitt D, Francois P, Vaudaux P, Foster TJ. Identification of the ligand-binding domain of the sur-
facing fibrinogen receptor (clumping factor) of Staphylococcus aureus. Mol Microbiol. 1995; 16: 895–907. PMID: 7476187

17. Hair PS, Echague CG, Sholl AM, Watkins JA, Geoghegan JA, Foster TJ, et al. Clumping factor A interaction with complement factor I increases C3b cleavage on the bacterial surface of Staphylococcus aureus and decreases complement-mediated phagocytosis. Infect Immun. 2010; 78: 1717–27. doi: 10.1128/IAI.01065-09 PMID: 20100856

18. Hair PS, Ward MD, Semmes OJ, Foster TJ, Cunnion KM. Staphylococcus aureus clumping factor A binds to complement regulator factor I and increases factor I cleavage of C3b. J Infect Dis. 2008; 198: 125–33. doi: 10.1086/588825 PMID: 18544012

19. Higgins J, Loughman A, Van Kessel KPM, Van Strijp JAG, Foster TJ. Clumping factor A of Staphylococcus aureus inhibits phagocytosis by human polymorphonuclear leucocytes. FEMS Microbiol Lett. 2006; 258: 290–6. PMID: 16640587

20. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. Genome sequence of Staphylococcus aureus strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J Bacteriol. 2008; 190: 300–10. PMID: 17951380

21. McAdow M, Kim HK, DeDent AC, Hendrickx APA, Schneewind O, Missiakas DM. Preventing Staphylococcus aureus sepsis through the inhibition of its agglutination in blood. PLoS Pathog. 2011; 7: e1002307. doi: 10.1371/journal.ppat.1002307 PMID: 22028651

22. Bae T, Schneewind O. Allelic replacement in Staphylococcus aureus with inducible counter-selection. Plasmid. 2006; 55: 58–63. PMID: 16051359

23. Kobayashi SD, Malachowa N, Whitney AR, Braughton KR, Gardner DJ, Long D, et al. Comparative analysis of USA300 virulence determinants in a rabbit model of skin and soft tissue infection. J Infect Dis. 2011; 204: 937–41. doi: 10.1093/infdis/jir441 PMID: 21849291

24. Kennedy AD, Wardenburg JB, Gardner DJ, Long D, Whitney AR, Braughton KR, et al. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis. 2010; 202: 1050–8. doi: 10.1086/656043 PMID: 20762702

25. Carson FL, Hladik C. Histotechnology: A self-instructional text. 3rd ed. American Society for Clinical Pathology Press; 2009.

26. Cheng AG, Kim HK, Burts ML, Krausz T, Schneewind O, Missiakas DM. Genetic requirements for Staphylococcus aureus abscess formation and persistence in host tissues. FASEB J. 2009; 23: 3393–404. doi: 10.1096/fj.09-135467 PMID: 19525403

27. Kwiecinski J, Jin T, Josefsson E. Surface proteins of Staphylococcus aureus play an important role in experimental skin infection. APMS. 2014; 122: 1240–50. doi: 10.1111/apm.12295 PMID: 25051890

28. Loof TG, Goldmann O, Naudin C, Mörgelin M, Neumann Y, Pils MC, et al. Staphylococcus aureus-induced clotting of plasma is an immune evasion mechanism for persistence within the fibrin network. Microbiology. 2015; 161: 621–7. doi: 10.1099/mic.0.000019 PMID: 25533444

29. Malachowa N, Kobayashi SD, Sturdavent DE, Scott DP, DeLeo FR. Insights into the Staphylococcus aureus-host interface: global changes in host and pathogen gene expression in a rabbit skin infection model. PLoS ONE. 2015; 10: e0117713. doi: 10.1371/journal.pone.0117713 PMID: 25719526

30. Miller LS, Cho JS. Immunity against Staphylococcus aureus cutaneous infections. Nat Rev Immunol. 2011; 11: 505–18. doi: 10.1038/nri3010 PMID: 21720387

31. Rogers DE. Studies on bacteremia: I. Mechanisms relating to the persistence of bacteria in rabbits following the intravenous injection of staphylococci. J Exp Med. 1956; 103: 713–42. PMID: 13319588
32. Rogers DE, Melly MA. Studies on bacteriemia: II. Further observations on the granulocytopenia induced by the intravenous injection of staphylococci. J Exp Med. 1957; 105: 99–112. PMID: 13406171
33. Cheung AL, Eberhardt KJ, Chung E, Yeaman MR, Sullam PM, Ramos M, et al. Diminished virulence of a sar/agr mutant of Staphylococcus aureus in the rabbit model of endocarditis. J Clin Invest. 1994; 94: 1815–22. PMID: 7962526
34. Lin Y-C, Anderson MJ, Kohler PL, Strandberg KL, Olson ME, Horswill AR, et al. Proinflammatory exo-protein characterization of toxic shock syndrome Staphylococcus aureus. Biochemistry. 2011; 50: 7157–67. doi: 10.1021/bi200435n PMID: 21749039
35. Spaulding AR, Lin Y-C, Merriman JA, Brosnahan AJ, Peterson ML, Schlievert PM. Immunity to Staphylococcus aureus secreted proteins protects rabbits from serious illnesses. Vaccine. 2012; 30: 5099–109. doi: 10.1016/j.vaccine.2012.05.067 PMID: 22691432
36. Strandberg KL, Rotschafer JH, Vetter SM, Buonpane RA, Kranz DM, Schlievert PM. Staphylococcal superantigens cause lethal pulmonary disease in rabbits. J Infect Dis. 2010; 202: 1690–7. doi: 10.1086/657156 PMID: 20979456
37. Crozier-Bertin D, Piroth L, Charles P-E, Larribeau A, Biek D, Ge Y, et al. Staphylococcus aureus leukocidin: A new virulence factor in cutaneous infections? An epidemiological and experimental study. Dermatology. 1992; 185:175-80. PMID: 1446082
38. Neves F, Abrantes J, Almeida T, de Matos AL, Costa PP, Esteves PJ. Genetic characterization of interleukin-1α (IL-1α), IL-1β, IL-4, IL-8, IL-10, IL-12A, IL-12B, IL-15 and IL-18) with relevant biological roles in lagomorphs. Innate Immun. 2015; 21: 787–801. doi: 10.1177/1753425915606209 PMID: 26395994
39. Mettas J, Hughes CCW. Of mice and not men: Differences between mouse and human immunology. J Immunol. 2004; 172: 2731–8. PMID: 14978070
40. Kielian T, Beardon ED, Baldwin AC, Esen N. IL-1 and TNF-α play a pivotal role in the host immune response in a mouse model of Staphylococcus aureus-induced experimental brain abscess. J Neurpathol Exp Neurol. 2004; 63: 381–96. PMID: 15099027
41. Holley MM, Kielian T. Th1 and Th17 cells regulate innate immune responses and bacterial clearance during central nervous system infection. J Immunol. 2012; 188: 1360–70. doi: 10.4049/jimmunol.1101660 PMID: 22190181
42. Cho JS, Guo Y, Ramos RI, Hebron F, Plaisier SB, Cian J, et al. Neutrophil-derived IL-1β is sufficient for abscess formation in immunity against Staphylococcus aureus in mice. PLoS Pathog. 2012; 8: e1003047. doi: 10.1371/journal.ppat.1003047 PMID: 22137885
43. Chan LC, Chaill S, Filler SG, Barr K, Wang H, Kupferwasser D, et al. Nonredundant roles of interleukin-17A (IL-17A) and IL-22 in murine host defense against cutaneous and hematogenous infection due to mephalin-resistant Staphylococcus aureus. Infect Immun. 2015; 83: 4427–37. doi: 10.1128/IAI.01061-15 PMID: 26351278
44. De Navasquez S. Experimental pyelonephritis in the rabbit produced by staphylococcal infection. J Pathol Bacteriol. 1950; 62: 429–36. PMID: 14784907
45. Freedman LR. Experimental pyelonephritis VI. observations on susceptibility of the rabbit kidney to infection by a virulent strain of Staphylococcus aureus. Yale J Biol Med. 1960; 32: 272–9. PMID: 13824718
46. Miller LS, Pietras EM, Urichio LH, Hirano K, Rao S, Lin H, et al. Inflammasome-mediated production of IL-1β is required for neutrophil recruitment against Staphylococcus aureus in vivo. J Immunol. 2007; 179: 6933–42. PMID: 17982084
47. Krishna S, Miller LS. Host—pathogen interactions between the skin and Staphylococcus aureus. Curr Opin Microbiol. 2012; 15: 28–35. doi: 10.1016/j.mib.2011.11.003 PMID: 22137885
48. Edens HAParks CA. Neutrophil transendothelial migration and alteration in vascular permeability: focus on neutrophil-derived azurocidin. Curr Opin Hematol. 2003; 10: 25–30. PMID: 12483108
49. Weisel JW, Fibrinogen and fibrin. In: Fibrous Proteins: Coiled-Coils, Collagen and Elastomers. Adv Protein Chem. 2005; 70: 247–99. PMID: 15837518