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Mouse models for infectious diseases caused by *Staphylococcus aureus*

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Abstract

Staphylococcus aureus - a commensal of the human skin, nares and gastrointestinal tract - is also a leading cause of bacterial skin and soft tissue infection (SSTIs), bacteremia, sepsis, peritonitis, pneumonia and endocarditis. Antibiotic-resistant strains, designated MRSA (methicillin-resistant *S. aureus*), are common and represent a therapeutic challenge. Current research and development efforts seek to address the challenge of MRSA infections through vaccines and immune therapeutics. Mice have been used as experimental models for *S. aureus* SSTI, bacteremia, sepsis, peritonitis and endocarditis. This work led to the identification of key virulence factors, candidate vaccine antigens or immune-therapeutics that still require human clinical testing to establish efficacy. Past failures of human clinical trials raised skepticism whether the mouse is an appropriate model for *S. aureus* disease in humans. *S. aureus* causes chronic-persistent infections that, even with antibiotic or surgical intervention, reoccur in humans and in mice. Determinants of *S. aureus* evasion from human innate and adaptive immune responses have been identified, however only some of these are relevant in mice. Future research must integrate these insights and refine the experimental mouse models for specific *S. aureus* diseases to accurately predict the failure or success for candidate vaccines and immune-therapeutics.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium that colonizes the skin, nares and gastrointestinal tract of humans (1). Approximately 20% of the human population are stably colonized while 30% are colonized in a variable manner (1, 2). *S. aureus* is also a pathogen that causes invasive disease, predominantly skin and soft tissue infections (SSTI) but also bacteremia, sepsis, pneumonia, osteomyelitis and endocarditis (1). The pathological hallmark of *S. aureus* infection is the formation of purulent abscess lesions that are formed around a nidus of the pathogen, primarily via the infiltration of neutrophils (3). In humans, *S. aureus* infection does not lead to the development of protective immune responses and chronic persistent or recurrent infections are common (4). Some isolates of *S. aureus* cause toxic-shock syndrome, exfoliative skin disease, and enteritis in humans (1). Secreted toxins

Competing interests: The authors declare a conflict of interests as inventors of patent applications that are related to the development of *Staphylococcus aureus* vaccines and are currently under commercial license.

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are the key virulence determinants for these diseases (5, 6) and transfer of the corresponding genes among staphylococcal strains involves specific bacteriophages (7).

S. aureus is also an important pathogen of live-stock, causing large scale infections in ruminants (sheep, goats, cows), poultry and pigs (8). Molecular epidemiological data suggest that a common pathogenic *S. aureus* clone associated with ruminants originated in humans (9). This strain adapted to its chosen niche more than 11,000 years ago, at a time when farming domesticated animals became common practice, and then diversified (9). Similar jumps from humans to new hosts occurred for several different clinical lineages (10). Adaptation to new hosts required a combination of gene loss, allelic diversification, and acquisition of mobile genetic elements, for example elements that support the expression of unique von-Willebrand factor binding protein alleles in *S. aureus* strains that infect ruminants and horses (11). Nevertheless, the core genome of ruminant associated *S. aureus* is stable and can lead to reciprocal transmission of newly emerging clones into the human population (12). This type of pathogen introduction occurs on a global scale and is associated with transport of live-stock or people (13). It has led to outbreaks of human *S. aureus* disease in countries that otherwise have low prevalence for staphylococcal disease (9).

The genome of *S. aureus* strains varies in size (2.6-2.9 MB), based on the presence of prophages and pathogenicity islands (14, 15). Nevertheless, all *S. aureus* isolates encompasses a core genome for the functional expression of genes that are shared by most if not all clinically relevant strains (16). For example, *S. aureus* isolates generally coagulate blood, agglutinate in plasma, and bind to animal immunoglobulin (17). Although nasal colonization is asymptomatic for most individuals, it represents a risk factor for hospital-acquired infection (18). Other risk factors for nosocomial infection include indwelling catheters, endotracheal intubation, medical implants, trauma, diabetes, immunosuppression and immunosuppressive therapy, hemodialysis and peritoneal dialysis (19, 20). In the United States, *S. aureus* is the single most frequent cause of hospital-acquired infectious disease mortality (21).

Massive use of antibiotics in animals and humans led to the selection of drug-resistant strains, designated MRSA for methicillin-resistant *S. aureus* (22). Broad spectrum β -lactam resistance is caused by *MecA*, a penicillin-binding protein that cannot be inhibited by β -lactamase resistant β -lactam or cephalosporin compounds (22). Recommended therapeutics against MRSA strains are vancomycin, daptomycin and linezolid (23-25). However, vancomycin-resistant (VRSA) strains, which acquired genes for the synthesis of altered peptidoglycan precursors from enterococci, have been isolated (26-28). *S. aureus* strains have also evolved resistance mechanisms to daptomycin and linezolid shortly after these compounds were approved for clinical use (28-30). MRSA infections occur frequently in American hospitals and in the community (21). The public health crisis of community- and hospital-acquired MRSA infections may be addressed through the development of vaccines, however all clinical trials that sought to address this problem have thus far failed (31). Healthcare providers currently have limited treatment options, a situation reminiscent of the pre-antibiotic era (32). The National Academy of Science's Institute of Medicine (IOM)

placed comparative effectiveness research on MRSA as one of the top 25 priorities for national investment. *S. aureus* was the only bacterial pathogen addressed in the IOM report.

***Staphylococcus aureus* infections of mice**

When designed to recapitulate human disease, animal studies with infectious agents aim to provide experimental proof for the molecular basis of pathogenesis, the establishment of protective immunity and the molecular mechanisms whereby immunity is achieved (33-35). Over the past forty years, infectious diseases research championed the mouse as a model for human infectious diseases, generating a plethora of reagents that enable rapid advances. Another important factor favoring the mouse is costs. Small body size, short gestation and large litter size render mice less expensive to rear than any other mammal currently used for infectious diseases research (35-37).

Mice are, however, not a natural host for human clinical *S. aureus* isolates and require genetic adaptation to cause transmissible disease in mouse facilities (38). Key clinical presentation of natural *S. aureus* infection is the development of preputial gland abscess lesions in male mice as well as nasal and gastrointestinal colonization (38). Because most experimental approaches are designed towards elucidating the virulence factors of human clinical isolates and deriving protective vaccines for humans, mouse isolates have not yet been examined in detail. Nevertheless, mice have served as the premier experimental model to study *S. aureus* blood-borne persistent and metastatic abscess formation (3), septic arthritis (39), sepsis (40), neonatal sepsis and meningitis (41), endocarditis (42), peritonitis (43), subcutaneous skin infection (44) and pneumonia (45). This work provided several key insights. First, *S. aureus* can induce a diverse spectrum of diseases in mice - similar to what is observed in humans - albeit that the infectious dose required to produce a desired pathology varies widely. Second, the genetic determinants of *S. aureus* required to produce specific pathologies vary between the different disease models but are generally consistent when analyzing different staphylococcal strains (3, 45). Third, some models require a very large inoculum to elicit disease pathology, and one cannot expect to identify many of the key genetic determinants of *S. aureus* that would support pathogenesis in humans, where a lower inoculum may suffice (46). Fourth, the pathogenesis of different *S. aureus* infectious diseases appears to require multiple virulence factors and these can differ between disease types (17, 47, 48). Fifth, some secreted factors play key roles in neutralizing human innate defenses and are expressed by virtually all human clinical isolates, yet these factors are not adapted to mice and play no role in the various disease models (49, 50). Sixth, both immune-competent inbred and outbred mice can be used as disease models although the infectious dose required to produce disease can vary by a factor of 10-100 (51).

Below we discuss the most frequently used mouse models for *S. aureus* disease, describe the key virulence factors, and the relevance of each model for human disease. We point to important mechanisms for pathogen survival, and describe how each model was used for the discovery of protective antigens. It has been argued that mouse models cannot accurately identify the protective antigens of *S. aureus* and that mouse studies have misinformed the development of vaccine antigens (52). This criticism is not entirely justified. Past vaccine

failures can also be explained by mismatches between disease model and trial design, by unexpected safety issues and by ignoring the immune evasive attributes of *S. aureus* (53).

Preparing *S. aureus* for experimental infection

Blood-borne spread of staphylococci can generate abscess lesion in any human organ tissue. Initially, *S. aureus* strains were isolated from specific organ tissues with the notion that unique genetic determinants or the variation of certain virulence genes may favor replication at these sites (54). For example, strains isolated from infected endocardium or infected joints were used to examine infective endocarditis and arthritis in animals, respectively (54). Extensive DNA sequence analysis of clinical isolates has demonstrated that *S. aureus* strains, although endowed with variable genetic traits (55), invariably retain the ability for blood-borne dissemination and for the seeding of abscess lesions (15, 16). Such insight enables the field to focus on a few well characterized strains, for example the methicillin-sensitive isolate *S. aureus* Newman (55) and the CA-MRSA isolate USA300 (LAC) (56), to conduct comparative analyses of virulence factors and vaccine antigens. To standardize the inoculation of staphylococci into animals, we recommend that bacteria are grown as overnight cultures in tryptic soy broth with rotation at 37°C, diluted 1:100 into fresh broth and incubated further to absorbance at 600 nm (A_{600}) 0.5. Staphylococci in culture aliquots should be sedimented by centrifugation, bacteria washed and suspended in sterile PBS to the desired volume and inoculum. Each inoculum should be quantified by spreading aliquots on agar plates and enumerating colony forming units (CFU).

Skin infection

Ogston first isolated *S. aureus* from the pus of surgical wound infections and showed that, when injected into the subcutaneous tissue of experimental animals, this material could elicit abscess lesions in guinea pigs and mice (57, 58). Infections in humans may occur at sites where the skin barrier function has been breached (wound or surgical site infections) or without apparent breaches, for example at hair follicles (folliculitis), as bullous or superficial lesions (impetigo), deep (furuncles) or confluent abscesses (carbuncles) (1). *S. aureus* infections of the human skin elicit purulent exudate, which drains from the infectious site. To induce skin infections in mice, the hair on the back or flank of mice is shaved and a suspension of 10^7 - 10^9 CFU *S. aureus* in PBS is injected into subcutaneous tissues (44, 59). Within 24 hours, bacteria elicit inflammatory responses and cause an indurated swelling, which increases over 5-7 days to a size of 30-100 mm² (59). Abscess lesions are gradually resolved over the next 7-9 days either with or without drainage of purulent material (44, 46). Depending on the *S. aureus* strain tested and its production of secreted α -hemolysin (α -toxin), subcutaneous skin lesions are associated with superficial dermonecrosis, which heals at a similar rate as the resolution of subcutaneous abscess lesions (46).

S. aureus strains require the structural gene for α -hemolysin (*hla*) to produce wild-type size lesions and dermonecrosis in the mouse model for skin disease (46). Immunization of mice with Hla_{H35L}, a non-toxic variant (60), elicits neutralizing antibodies that provide protection against Hla-mediated pathological effects but cannot prevent the establishment of smaller sized lesions without dermonecrosis (46). Similar protective effects are achieved in

passive transfer experiments with monoclonal antibodies that neutralize Hla (61). ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) serves as the receptor for Hla and is conserved in humans and mice (62). The Hla-ADAM10 interaction leads to disruption of host cell membrane junctions, a process that exacerbates necrosis of the epidermis and dermis (46, 62, 63).

MRSA strains of the USA300 lineage are causing an epidemic of community-acquired skin infections in the United States (64, 65). These strains express a bacteriophage encoded leukocidin, the pore-forming cytotoxin PVL (66, 67), which binds to the complement receptors C5aR and C5L2 of human leukocytes but not to the complement receptors of murine immune cells (68). Not surprisingly, deletion of the structural genes for PVL does not affect the pathogenesis of USA300 variants in the mouse skin infection model (69, 70).

Phenol soluble modulins (PSMs) contribute to the pathogenesis of mouse skin infections. Originally identified in *S. epidermidis* (71), subsequent studies in MRSA described two types of PSMs: α -PSMs are relatively short (20-25 amino acid long) and form an amphipathic α -helix, whereas β -PSMs are longer (~50 amino acid long) and only their C-terminal domain may adopt an α -helical structure (72, 73). PSMs are not synthesized as signal-peptide bearing precursors and require a dedicated ATP-binding cassette (ABC) transporter for secretion as formylated peptides (74). PSMs elicit proinflammatory responses by interacting with the formyl peptide receptor 2 (FPR2) of host cells including neutrophils (74, 75). In particular, PSM α 3 has been proposed to activate, attract and lyse neutrophils (72). *S. aureus* continues to produce PSMs following its uptake by neutrophils, suggesting that PSMs may perhaps promote an intracellular lytic activity (76). In agreement with these hypotheses, *S. aureus* variants unable to produce α -PSMs are associated with reduced dermonecrosis (72, 74).

The mouse model for *S. aureus* skin infection has been modified to examine the contribution of specific skin cells or tissue structures and of specific immune cells such as neutrophils or macrophages. Some protocols damage the skin (removal of superficial keratinocytes, incision or heat damage) prior to inoculation of *S. aureus*, whereas other protocols inoculate staphylococci along with implanted foreign material (sutures, dextran beads, cotton dust) (77-80). These modifications allow investigators to reduce the staphylococcal challenge dose required to cause pathologic lesions.

Depletion of immune cells via specific monoclonal antibodies (anti-Ly6G) or chemotherapy (cyclophosphamide) causes a dramatic reduction in the challenge dose required for skin infection (81, 82). In contrast to immune-competent mice, leukopenic mice cannot contain staphylococci in the skin and develop systemic infections with rapidly lethal outcome (83). Similar observations have been made in humans, where iatrogenic leukopenia or hereditary defects in the NADPH oxidase or in the respiratory burst of myeloid cells are associated with increased susceptibility towards *S. aureus* infection (84). Humans with abnormal T cell function (including HIV/AIDS patients), atopic dermatitis, and hyper-IgE syndrome, also display increased susceptibility toward *S. aureus* cutaneous infection (85-88). These phenotypes can be recapitulated in mice with similar skin conditions or immune defects (80). For example skin lesions of *S. aureus*-infected MyD88- and IL-1R-deficient mice, and

to a lesser extent TLR2-deficient mice, are substantially larger in size, with higher staphylococcal load, as compared to lesions in wild-type mice (89). The severity of skin infections correlates with the decreased recruitment of neutrophils to the site of infection (89). Similarly, mice deficient in dendritic epidermal T cells ($\gamma\delta$ T cells) and IL-17R develop larger skin lesions with higher bacterial load and impaired neutrophil recruitment (90). IL-17 producing T lymphocytes (T_H17) play an important role in controlling *S. aureus* cutaneous infection by recruiting neutrophils to the infectious site (91). A growing body of evidence implicates T_H17 cells as critical for protection against skin and lung infections, whereas T_H17 /IL-17 responses may be less critical in other tissues (92, 93).

Thus, immune-competent mice (C57BL/6 or BALB/c) represent a good model for the study of *S. aureus* skin infections and the characterization of staphylococcal virulence factors. The subcutaneous injection of a relatively large inoculum prevents the identification of virulence factors acting at early stages of disease pathogenesis (adherence to skin structures, invasion across epidermal layers). As some secreted factors of *S. aureus* require human specific interactions, the mouse model can likely be improved by generating transgenic animals expressing specific human genes or by developing mouse models in which the mouse innate or adaptive immune system is replaced by its human counterpart (94). For example, expression of human complement receptors C5aR in murine myeloid cells could restore susceptibility toward staphylococcal PVL. Approximately one-fifth of SSTI patients receiving antibiotic therapy develop recurrent skin infections with the same strain (95). It will be important to determine whether recurrent skin infection occurs in mice and, if so, what genetic determinants of staphylococci promote this disease process.

Bacteremia and metastatic abscess formation

Invasive staphylococcal disease in humans is associated with staphylococcal bacteremia and the formation of abscess lesions in many different organ tissues (96). In mice, intravenous inoculation of *S. aureus* also triggers dissemination of blood-borne bacteria into organ tissues, where they establish abscess lesions in skeletomuscular, vascular, brain, lung, heart, liver, spleen and kidney tissues (3). Briefly, mice are injected into the tail vein or the periorbital venous plexus with 10^6 - 10^7 CFU, which causes staphylococcal bacteremia (3). The bacteria eventually exit from the blood stream but can be recovered from peripheral organs, for example renal tissues, where they attract large immune cell infiltrates. Hematoxylin-eosin staining of thin-sectioned tissue samples provides the first histopathologic evidence for *S. aureus* infection, as early as 48 hours following intravenous inoculation (3). By day 4, renal tissues harbor multiple abscess lesions that, on histopathologic examination are characterized by a bacterial nidus (*staphylococcal abscess community*) encapsulated by eosinophilic fibrin deposits. These fibrin deposits shield staphylococci from surrounding immune cell infiltrates. During early stages of abscess development (days 2-5), immune cells infiltrates are comprised predominantly of neutrophils, arranged in concentric layers of apoptotic, healthy and apoptotic PMNs (3). Macrophages and lymphocytes are positioned in the periphery of these lesions (97). By day 15 following inoculation, T and B lymphocytes as well as macrophages are recruited into abscess lesions, which slowly progress towards the organ surface and eventually rupture to release purulent material with staphylococci into circulation (3). Released staphylococci

seed new abscesses at other sites and, by day 30-50, accumulating lesions eventually promote lethality (3).

S. aureus Newman variants have been analyzed for their phenotypic defects in the renal abscess model. Sortase A mutants, defective for anchoring any one of 19 surface proteins with LPXTG sorting signals to the cell wall envelope, are unable to cause abscess lesions (3). In contrast, mutations in individual surface protein genes cause either no significant defect or display a reduction in the number and size of abscess lesions and in staphylococcal load (3). Mutants that display the most severe defects harbor mutations in genes for the following surface proteins: staphylococcal protein A (SpA), iron-regulated surface determinant A (IsdA) and B (IsdB), adenosine synthase A (AdsA), as well as serine-aspartate repeat protein D (SdrD) (3). Both IsdA and IsdB promote heme-iron scavenging during infection (98, 99) and antibodies against these proteins provide partial protection by reducing the number of abscess lesions and the bacterial load in renal tissues (100-102). The IsdB vaccine was subjected to a clinical trial in humans to reduce post-surgical mediastinal and superficial wound infections as well as bacteremia in patients undergoing cardiothoracic surgery (103). The trial was ended because multiorgan dysfunction and mortality following *S. aureus* infection occurred more frequently in individuals receiving the IsdB vaccine than in control cohorts and IsdB immunization did not show clinical benefit (103). Antibodies against SdrD also provide partial protection against *S. aureus* renal abscess formation, albeit that the molecular basis for protection is not known since the exact function of SdrD is still unknown (101). A similar protective effect is achieved when the CnaB domain of SdrE, which is conserved among members of this protein family (SdrD and SdrE) (104), is used as a vaccine antigen (105).

SpA binds to human and animal immunoglobulins (IgG, IgA, IgD, IgE and IgM) either at Fc γ , the C_H2-C_H3 hinge region of IgG involved in binding to complement factor C1q and Fc γ -receptors, or the heavy chain variable region (Fab) of V_H3 class antibodies (106-108). The former activity neutralizes antibody effector functions for opsonophagocytosis (109), whereas the latter activates V_H3-clan B cell receptors (IgM), resulting in non-productive B cell expansions and suppression of adaptive immune responses (110, 111). SpA-neutralizing polyclonal or monoclonal antibodies provide partial protection in the renal abscess model but also neutralize the B cell superantigen activity of staphylococci to promote antibody responses against almost all secreted products of *S. aureus* (112, 113). When used as a vaccine antigen, SpA_{KKAA}, a non-toxigenic variant that cannot bind immunoglobulin, elicits neutralizing antibodies and partial protection against *S. aureus* renal abscess disease (112). Further, SpA_{KKAA} immunization enhances antibody responses against many secreted products of *S. aureus* in animals that are exposed to this pathogen (112). SpA-mediated B cell superantigen activity is thought to suppress pathogen-specific antibody responses and the development of protective immunity (114). In agreement with this, infection of mice with *spa* variants that lack B cell superantigen activity elicits broad-spectrum antibody responses and promotes protective immunity (111).

AdsA, another surface protein requiring sortase A for envelope assembly, catalyzes the conversion of ATP, ADP and AMP to adenosine, a signal for mammalian immune suppression (115, 116). AdsA also converts 5' and 3' monophosphate cleavage products of

DNA, derived from staphylococcal nuclease activity with NETs (neutrophil extracellular DNA traps) (117), into deoxyadenosine, thereby inducing apoptosis in macrophages (97). This mechanism supports abscess formation and staphylococcal escape from phagocytic clearance in these lesions, which for several days remain devoid of macrophages (97).

S. aureus secretes two polypeptides, coagulase (Coa) and von-Willebrand Factor binding protein (vWbp), that associate with and activate prothrombin to generate staphylothrombin complexes, thereby cleaving fibrinogen and generating fibrin clots (118, 119). This activity, which can be monitored *in vitro* with the coagulase test, is essential for the establishment of renal abscesses, as *coa/vwb* mutants are unable to generate these lesions (119). When used as vaccine antigens, Coa and vWbp raise neutralizing antibodies that protect against abscess lesions (119). Of note, α -hemolysin does not play a key role in the establishment of abscess lesions (3). Capsular polysaccharides (CP5/CP8) and poly-*N*-acetylglucosamine (PNAG) have been advocated as important vaccine antigens, raising antibody responses protective against staphylococcal abscess formation in renal tissues (120-123). Compared with other virulence determinants, the traits for capsule or PNAG production are not critically important for disease pathogenesis in *S. aureus* Newman and in the CA-MRSA isolate USA300 LAC, presumably because encapsulation is either diminished or absent (3, 124).

In summary, staphylococcal abscess formation can be thought of as a developmental process that occurs in four discrete stages (17). Following intravenous inoculation, *S. aureus* requires specific genes to survive in the blood stream of infected mice for subsequent deposition into peripheral organ tissues (Stage I). Of note, staphylococci can disseminate as extracellular pathogens but also when located within neutrophils (125). Staphylococcal invasion of host tissues triggers massive immune cell infiltrations with neutrophils predominating early during infection (Stage II). Due to the formation of a fibrin capsule and other virulence strategies, staphylococci replicate as abscess communities and manipulate immune cell infiltrates to promote tissue destruction and abscess maturation at organ surfaces (Stage III). Liquefaction necrosis and drainage of pus releases staphylococci for renewed dissemination to new sites within the infected host (Stage IV).

Blood stream infections are a risk factor for septic arthritis in humans and the metastatic abscess model can also be used to study *S. aureus* septic arthritis in mice (54). Following inoculation of staphylococci, mice are weighed daily and examined for arthritis and clinical appearance. At timed intervals, one pair of limbs (fore and hind) is used for histopathologic examination of joints (synovitis), while paws are examined for the bacterial load. Alternatively, bacteria may be injected directly in the knee joint (126). In this model, 3.6×10^4 CFU of strain Newman in 20 μ l of PBS are injected into the joint and animals are killed 3 days after inoculation for histopathologic examination of synovitis. Synovitis is defined as synovial membrane thickness of over two cell layers and infiltration of inflammatory cells to the extra-articular space with cartilage or bone destruction (126). A histopathology index scores the severity of synovitis and tissue destruction (126). *S. aureus* sortase A, protein A and clumping factor A (*vide infra*) are virulence factors in this model, whereas Hla and IsdA/IsdB are not (127-131).

Sepsis

S. aureus is a common cause of sepsis, a frequently fatal, systemic inflammation with multiple organ dysfunction, typically triggered by immune responses towards bacterial replication in blood (132). Intravenous inoculation of 5×10^7 - 5×10^8 CFU *S. aureus* causes infected animals to develop septic shock with lethal outcome within 12-48 hours (119). Animals present with clinical signs of disease within 2-3 hours, including ruffled fur, diminished activity and appetite, hunched posture, loss of movement, diarrhea, dehydration and labored breathing (3). During necropsy, staphylococci can be isolated from blood and from all organ tissues examined. *S. aureus* Newman variants lacking sortase A are avirulent in this model system and do not precipitate a lethal outcome over 14 days following blood stream inoculation (133). Two surface proteins play a key role in sepsis pathogenesis, AdsA (*vide supra*) and ClfA (133). Clumping factor A (ClfA) binds to the D domain of fibrinogen and fibrin (134-136). When expressed in human or animal plasma together with Coa and vWbp, ClfA mediates staphylococcal association with fibrin filaments, a phenotype that has been designated clumping or agglutination (133). ClfA association with fibrin filaments protects staphylococci from macrophages (137) and also promotes vascular occlusions by agglutinated bacteria in organ tissues (133). A triple mutant strain lacking *coa*, *vwb* and *clfA* is avirulent in the mouse sepsis model, whereas *clfA* or *coa/vwb* variants are severely attenuated (119, 133). The pathogenesis of *S. aureus* sepsis can be perturbed with antibodies that neutralize the biochemical activities of Coa, vWbp and ClfA or with direct thrombin inhibitors, small molecules that also block staphylothrombin cleavage of fibrinogen (119, 133). ClfA, Coa and vWbp-derived vaccines can prevent *S. aureus* sepsis in mice (138) and mice with a genetic defect in fibrinogen, which abolishes its association with ClfA, are less susceptible to *S. aureus* induced sepsis than wild-type mice (139).

S. aureus hla mutants display delayed time-to-death and increased survival in the mouse sepsis model, likely because ADAM10 activation by the toxin is a key factor for the increased vascular permeability associated with disease (140). *S. aureus* secretes several other pore forming toxins, LukED and LukAB, that cause injury to myeloid cells by interacting with their G-protein coupled receptor molecules (141-144). The severity of *S. aureus* induced sepsis can be ameliorated by depletion of neutrophils that are targeted by LukED in a manner requiring specific chemokine receptors (141, 145). LukAB association with its CD11b receptor occurs in a host specific manner, as this toxin is capable of injuring human neutrophils but not mouse neutrophils (144). The possibility of targeting leukocidins (LukED, LukAB, PVL) as vaccine antigens represents an exciting frontier that awaits the development of refined mouse models with functional human chemokine receptors on myeloid cells.

Peritonitis

S. aureus is a frequent cause of peritonitis in individuals with end-stage renal disease and continuous ambulatory peritoneal dialysis (146). When injected with staphylococci into the peritoneal cavity, mice require a very large inoculum to develop lethal disease: $LD_{50} = 5 \times 10^8$ CFU and $LD_{90} = 6 \times 10^9$ CFU for *S. aureus* Newman (43). The animals typically succumb within 12-24 hours of challenge and survivors of lower challenge doses harbor

intraperitoneal abscess lesions filled with large numbers of immune cells, mostly neutrophils and macrophages, and surrounded by layers of fibrin and collagen (43). Very few staphylococci enter the blood stream and the establishment of metastatic abscess lesions is rare. On the other hand, lesions that were formed within the recesses of the peritoneal cavity are often associated with tissues of organs that are covered with peritoneal lining, e.g. the kidneys. Following necropsy, staphylococci are found in homogenized renal tissues, although the bacteria were located in the peritoneal cavity but not in renal parenchyma (43). The ease of administering staphylococci into the peritoneal cavity has led to the widespread use of this model and the identification of capsular polysaccharides as a key virulence determinant (147). Several studies indicate however that α -toxin is the key determinant for the lethal outcome of intraperitoneal *S. aureus* challenge (43, 148). In agreement with this, active immunization with Hla_{H35L} or passive immunization with Hla-neutralizing antibody protects mice against the lethal outcome of intraperitoneal *S. aureus* inoculation but does not affect intraperitoneal abscess formation (43, 149).

Pneumonia

S. aureus infection of the lower respiratory tract leads to significant morbidity and mortality (150). Disease severity correlates with staphylococcal isolates in particular CA-MRSA strains (151). *S. aureus* is also a frequent cause of ventilator-assisted pneumonia in patients with significant co-morbidities (152). Instillation of staphylococcal suspensions (4×10^8 CFU *S. aureus* Newman) into the left nare of anesthetized adult mice that are held upright causes labored breathing marked by a high respiratory rate and exaggerated chest wall excursion immediately after infection (45). This state resolves within 6 h, however the combined effects of aspiration and *S. aureus* virulence leads to the development of pneumonia with 50% mortality at 24 h, followed by an additional 20% of the animals succumbing within 48 h following inoculation (45). At these time points, all infected animals appear ill, and have an increased respiratory rate, hunched posture, and decreased mobility. A small reduction of the inoculum (8×10^7 CFU) results in no mortality, although all animals appear ill but recover fully within 48 h. Inoculation with 8×10^8 CFU of *S. aureus* Newman results in about 90% mortality by 24 h. For bacterial enumeration (CFU) and histopathology analysis of lungs, animals may be infected with 2×10^8 CFU of *S. aureus* (45). Histopathology analysis reveals infiltration of immune cells and destruction of alveolar architecture by 24 h post challenge. These damages correlated with increased replication of staphylococci (45). Animals that survive beyond the 72 h observation period, resolve the infection, show lungs with restored air space and only residual inflammation on alveolar walls (45). α -hemolysin is the key virulence factor for the pathogenesis of *S. aureus* pneumonia in this model, whereas surface proteins and other secreted product play either no specific or minor roles (45, 60). Mutant mice that lack expression of the ADAM10 receptor for α -hemolysin display resistance towards *S. aureus* pneumonia (63). Both active (Hla_{H35L}) and passive immunization of mice with neutralizing antibodies provides complete protection against lethal pneumonia following challenge with both MSSA and MRSA strains (60, 153). In a pneumonia model with neonatal mice, SpA has been shown to function as a virulence factor (154). In this model, SpA activates tumor necrosis factor receptor 1 (TNFR1) and epidermal growth factor receptor (EGFR) signaling cascades, thereby perturbing tight junctions of alveolar epithelia

(155, 156). Compared to wild-type *S. aureus*, a *spa* mutant fails to transmigrate the epithelial monolayer in tissue culture media (157). *tnfr-1* null mice were found to be more resistant to pulmonary infection as compared to wild-type animals, providing support for a distinct host pathogen interaction mediated by SpA (158).

Models requiring surgery

S. aureus frequently infects medical implants and catheters by forming biofilms on the surfaces of these inert structures (1, 159). Several different mouse models involving *S. aureus* infection of surgically implanted medical devices and catheters have been developed (160-163). Because of space constraints, we must limit the discussion below to two mouse models involving surgery: staphylococcal osteomyelitis and endocarditis.

Osteomyelitis or musculoskeletal infection of children occurs with an annual incidence of 1 in 5,000-10,000 in the United States (164, 165). In adults, osteomyelitis is associated with compound fractures, contiguous soft-tissue infections, and diabetes (166). As with other staphylococcal infections, CA-MRSA is a causative agent of osteomyelitis in otherwise healthy individuals. *S. aureus* inflicts significant bone destruction and local inflammation, and, if not controlled, disseminates into the blood stream. Thus, treatment of osteomyelitis involves surgical debridement followed by prolonged antimicrobial therapy and is complicated by bacteremia, venous thrombosis, and pathologic fractures (167). A mouse model of osteomyelitis was recently described whereby a 2- μ l suspension containing 10^6 CFU of *S. aureus* is inoculated into the intramedullary canal of the femurs of animals following the creation of a 1-mm unicortical bone defect (168). For this procedure, a small incision is made to expose the left femur of the hind limb of anesthetized animals, and trephination with a 21-gauge needle is performed to create a 1-mm diameter unicortical bone defect at the mid-femur. Bacteria are delivered through the bone defect into the intramedullary canal. All exposed areas are immediately closed with suture, and mice administered buprenorphine every 12 h for 72 h following surgery (168). Disease has been assessed by CFU enumeration upon removal of femur. Cassat and colleagues also used high-resolution microcomputed tomography and histology for visualization of bone loss and abscess formation (168). Continuous observation over 14 days shows that the staphylococcal burden peak at day 4 and imaging analysis of microcomputed tomography reveals profound bone remodeling (both destruction and formation) at the damaged site. Infected femurs lose up to 50% of their original cortical volume. Mock-infected animals heal rapidly following surgery. Histopathology examination demonstrates substantial abscess formation throughout the bone marrow of infected femurs. A mutant lacking *sae* was found to be attenuated in this model (168). The *sae* locus encodes a two component regulatory system that controls the secretion of numerous proteins in *S. aureus*. The Sae-regulated protease aureolysin was found to be a key contributor for this disease by mediating the degradation of PSMs, which in turn triggered osteoblast cell death and bone destruction (168). Of note, aureolysin is not a virulence factors for *S. aureus* induced septic arthritis (169).

Infective endocarditis (IE) occurs in 30-60% of patients with *S. aureus* bacteremia and is associated with 40-50% mortality (170). IE is characterized by the formation of septic vegetations on the endocardium that consist of a meshwork of host factors, such as fibrin

and platelets, as well as bacterial aggregates (171). Currently, *S. aureus* is the leading causative agent of infective endocarditis, a rapidly progressing, destructive infection of the heart valves (172, 173). An increased frequency of nosocomial endocarditis caused by *S. aureus* has also been documented, possibly linked to increased use of intravascular devices (172). It has also been reported that *S. aureus* bacteremia in patients with intravascular devices often results in venous thrombosis (174). A robust animal model of *S. aureus* endocarditis should feature aortic valve bacterial vegetations resembling those in humans. These features have been achieved in a mouse model of experimental infective endocarditis, which encompasses intravenous challenge with 10^6 CFU *S. aureus* one day following aortic valve trauma (175). Valve trauma is produced by introduction of an indwelling 32-gauge polyurethane catheter (sealed) into the aortic valve via the left carotid artery on anesthetized animals (175). Animals are placed in dorsal recumbency, the hair on the ventral neck and chest region shaved, and the shaved skin cleaned. Animals are kept on a vented warming table for thermoregulation and an incision is made from the thoracic inlet to the ramus of the left mandible. The left carotid artery is isolated and ligated cranially with suture. A surgical microscope is used to produce a small hole in the carotid artery with a 27-gauge needle to insert the sealed tube (catheter) that is fed retrograde through the aortic valve (for a length of 1.3-cm). The catheter is secured in place by sutures and the incision closed. Catheters are left in place for the duration of the study and mice are infected 18 to 24 h following this procedure (175). The model has been validated by using a vancomycin regimen at exposures comparable to human dosing (175). It has also been used to demonstrate the essential contribution of staphylocoagulases, Coa and vWbp, to IE (42). Because of the small size of the carotid artery, the mouse is a technically challenging model for the study of IE, which can also be examined in rats or rabbits (176, 177).

Conclusions and Perspectives

Mouse models have been essential for the study of several different infectious diseases caused by *S. aureus*, revealing virulence determinants and supporting efficacy studies with vaccine antigens or immune therapeutics. This work demonstrated also that *S. aureus* infection does not elicit protective immune responses against subsequent challenge with the same strain or with different *S. aureus* isolates (112, 178). These insights initiated a search for immune evasive factors that may represent key targets for vaccine development, however only some of these targets can currently be studied in mice (114). These limitations may be addressed by developing transgenic animals that express human factors critical for disease pathogenesis, as has been achieved for IsdB-mediated heme-iron scavenging in mice transgenic for human hemoglobin (179, 180). Assessing the contributions of several other human-specific virulence factors, for example SCIN, CHIPS, and SAK, which directly or indirectly inhibit C3 convertase, C5a receptor, and C3b, will require development of new transgenic models (49, 181). Another key insight is the realization that different types of diseases (sepsis, abscess formation, peritonitis, osteomyelitis, etc.) require different types of virulence factors. Thus, if key virulence determinants were chosen as vaccine antigens, single antigen vaccine approaches cannot be expected to address all of the staphylococcal diseases discussed herein. A universal vaccine, derived from *S. aureus* virulence factors, would need to incorporate several different antigens into a single vaccine formulation (3,

101, 119, 178, 182). Finally, mouse models, developed for any one *S. aureus* disease, can reveal virulence contributions and vaccine efficacy of a specific antigen(s), and this information may be useful to predict the success of human clinical trials for the same disease. It seems unlikely, however, that such data may predict the success of clinical trials addressing another disease. If so, studies with capsular polysaccharide vaccines in the mouse peritonitis model cannot predict vaccine success in patients with end-stage renal disease and hemodialysis-related bacteremia (123, 183). Further, studies with IsdB in mouse models for sepsis or abscess formation are unlikely to predict IsdB vaccine success in patients with mediastinal infections following cardiothoracic surgery (102, 103). We presume that mouse models for *S. aureus* diseases will remain the most important surrogates for the study of staphylococcal infections in humans, their therapy and prevention.

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Highlights

- *Staphylococcus aureus* is a human commensal
- *S. aureus* causes invasive and recurrent diseases
- *S. aureus* infections fail to elicit protective immune responses
- *S. aureus* disease attributes can be studied in experimental mouse models of infection

Figure 1.

Mouse models for *S. aureus* infections. Mice are susceptible to *S. aureus* infections. Staphylococci can be administered by four different routes: intravenous, intraperitoneal, subcutaneous and intranasal inoculation. Intravenous delivery of staphylococci generates metastatic infectious lesions in multiple internal organs including heart and kidneys. In heart, agglutinated staphylococci (staphylococcal abscess community; SAC) are surrounded by necrotic cardiac myocytes (red). In renal tissue, SAC are surrounded by fibrin deposits (brown - eosinophilic pseudocapsule) which separate bacteria from massive immune cell infiltrates (purple). Injection of staphylococci into the peritoneum creates a lesion comprised of a large number of immune cells with staphylococci. This lesion is attached to the peritoneal lining and surrounded by an inner layer of fibrin deposits (brown) and an outer layer of collagen (blue). Subcutaneous injection of staphylococci generates a subcutaneous abscess and dermonecrotic (red) lesions on the overlying skin. Lower respiratory tract infection caused by intranasal inoculation of staphylococci is characterized by obstruction of airspace (red) by inflammatory cell infiltrates and aggregates of *S. aureus*.

Table I.Summary of mouse models for *S. aureus* infection

Disease model	Infection route	Infectious dose (CFUs)	Phenotype	References
Skin infection	Subcutaneous	$1 \times 10^7 - 1 \times 10^9$	Dermonecrosis caused by secreted toxins	(46, 59)
Metastatic abscess formation	Intravenous	$1 \times 10^6 - 1 \times 10^7$	Abscess formation in most internal organs	(3)
Sepsis	Intravenous	$5 \times 10^7 - 5 \times 10^8$	Acute lethal disease within 48 hours of infection; formation of multiple lesions in heart	(119, 133)
Peritonitis	Intraperitoneal	5×10^8 (LD ₅₀) 6×10^9 (LD ₉₀)	Acute lethal disease within 12 hours of infection; formation of abscess lesions on peritoneal surfaces	(43)
Pneumonia	Intranasal	$2-4 \times 10^8$	Acute lethal disease within 72 hours of infection; infiltration of inflammatory cells into alveolar air space	(45)