OPINION

Models matter: the search for an effective *Staphylococcus aureus* vaccine

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Abstract | *Staphylococcus aureus* is a highly successful bacterial pathogen owing to its abundance of cell surface and secreted virulence factors. It is estimated that 30% of the population is colonized with *S. aureus*, usually on mucosal surfaces, and methicillin-resistant *S. aureus* is a major public health concern. There have been multiple attempts to develop an *S. aureus* vaccine using one or more cell surface virulence factors as antigens; all of these vaccine trials have failed. In this Opinion article, we suggest that an over-reliance on rodent models and a focus on targeting cell surface components have been major contributing factors to this failure.

Vaccination is widely recognized as one of the most cost-effective public health interventions. Early vaccine development efforts that targeted bacterial pathogens resulted in vaccines for *Clostridium tetani*, *Corynebacterium diphtheriae* and *Bordetella pertussis*, and later efforts resulted in vaccines that targeted *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. Most of these vaccines involved a single antigenic target — either a toxin or the capsule — that, when targeted, provided immune protection. The development of vaccines for most remaining bacterial pathogens, including *Staphylococcus aureus*, is more difficult, as the pathogenesis of these organisms is multifaceted.

S. aureus is the most important cause of serious infectious diseases, such as toxic shock syndrome (TSS), pneumonia, sepsis and infective endocarditis (FIG. 1), in the United States[1]. The life-threatening illnesses that are associated with *S. aureus* infection are dependent both on cell surface and on secreted virulence factors[2,3]. As the emergence of multidrug-resistant *S. aureus* strains is predicted to continue, efforts have focused on disease prevention via vaccination or immunotherapeutic approaches. As a minimum, it is desirable to develop vaccines that protect against life-threatening *S. aureus* infections, but it is hoped that vaccines could be developed that protect against all *S. aureus* infections, including the many soft tissue infections, such as abscesses and surgical site infections, that are associated with *S. aureus*. The vaccine approaches that have been used so far have all involved targeting cell surface components, including the polysaccharide capsule, the extracellular polysaccharides that are important for biofilm formation, the cell wall-associated proteins that recognize adhesive matrix molecules and aid in surface attachment and colonization, or ATP-binding cassette (ABC) transporters, which have a role in nutrient uptake and drug resistance. However, none of these immunization strategies has succeeded in human trials (BOX 1). The latest *S. aureus* vaccine failure[4] emphasizes the need to address the reasons for these failures and provides an opportunity to correct them.

Recent discussions on the failure of staphylococcal vaccines have focused on our lack of understanding of *S. aureus* pathogenesis in humans[5–7], on the large range of virulence factors that are produced by *S. aureus* (many of which have redundant functions) and their contributions to *S. aureus* pathogenesis[5,8–11], as well as on the nature of protective immunity against *S. aureus* infection[8,12–14]. There seems to be a consensus among investigators that the multiplicity and redundancy of *S. aureus* virulence factors creates an enormous challenge for the development of an effective vaccine and that the failure of vaccine development so far is a result of not knowing the correct combination of antigens or of using a range of antigens that is too narrow[7,9,10]. Furthermore, some argue that not knowing the correlates of immune protection is the greatest obstacle to vaccine development[11,13,14]. In addition, the protective role of antibodies in immune defence against *S. aureus* infections has also been questioned, and it has been argued that opsonic *S. aureus*-specific antibodies already exist in humans and are not sufficient to provide protection from infection, and an antibody-based vaccine is therefore unlikely to succeed[14,15].

More than 100 years have passed since the discovery of *S. aureus* and the diseases that it causes, and we still know relatively little about *S. aureus* pathogenesis[3]. As Projan *et al.* wrote, "little knowledge is a dangerous thing"[5]. However, the development of useful vaccines and immunotherapeutic agents to target *S. aureus* infections depends on a correct understanding of the molecular pathogenesis of *S. aureus*. This requires the use of appropriate animal models. Mice have been the animal models of choice for most pathogenesis studies, owing to their low cost. However, the most important consideration when selecting a model system for preclinical studies should be whether or not it closely mimics human infections. So far, mouse models of *S. aureus* infection have failed to predict human outcomes in clinical trials[8,12]. Although many investigators concede that mouse models are imperfect[5–7,10,12,14], in practice, most investigators continue to dismiss these inadequacies and still expect these models to shed light on crucial aspects of *S. aureus* pathogenesis[16] and mechanisms of immune protection against *S. aureus* infection[8,17].

It is also crucial to recognize that *S. aureus* causes serious illnesses, most notably menstrual and non-menstrual TSS[18], lethal pneumonia[19], infective endocarditis

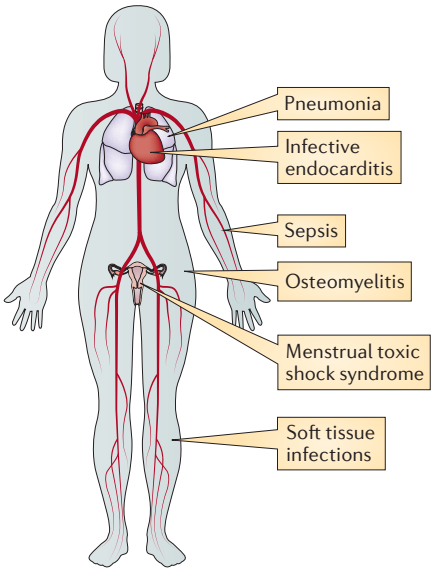

Figure 1 | **Diseases caused by *Staphylococcus aureus*.** *S. aureus* causes life-threatening diseases, including pneumonia, infective endocarditis, toxic shock syndrome and sepsis, as well as minor diseases such as soft tissue infections.

and sepsis[20], via massive immune system activation and dysregulation, and the lethality of these diseases is dependent on the effects of superantigens (SAgs), such as TSS toxin 1 (TSST1), staphylococcal enterotoxin B (SEB), SEC, and cytotoxins, such as α-toxin, on the cardiovascular system (reviewed in REF. 19). Physiologists recognize that the cardiovascular physiology of mice does not resemble that of humans[21], and in our opinion, this is a major drawback of mouse models of *S. aureus* infection. By contrast, the cardiovascular physiology of rabbits closely resembles that of humans[22,23]. Rabbits and humans also respond similarly to SAgs and cytotoxins, whereas mice respond differently[24]. Although rabbits are more difficult to work with, owing to the cost, the lack of inbred strains and greater government regulatory control, their immune and cardiovascular systems respond to *S. aureus* infection in a manner that is more comparable with that of humans.

In this Opinion article, we discuss the soundness of targeting *S. aureus* surface components, compared with secreted factors, as a vaccine strategy, as well as the use of mouse models to study *S. aureus* pathogenesis and vaccine outcomes.

Targeting the *S. aureus* surface

Most *S. aureus* vaccines or immunotherapies that have been tested or that are in development target surface components. The failure of the V710 vaccine (Merck) (which targeted the cell wall-anchored iron-responsive surface determinant B (IsdB)), in which vaccinated patients who developed an *S. aureus* infection were fivefold more likely to die than unvaccinated patients who developed an *S. aureus* infection[4], took the field by surprise, and investigators have struggled to make sense of these findings.

However, it is well-established that cell surface virulence factors facilitate host colonization via a combination of cell attachment and avoidance of the innate immune system. *S. aureus* uses a range of these virulence factors (for example, fibronectin-binding proteins and clumping factors) to aggregate, forming large bacterial clumps that are protected by a capsule and 'decorated' with host factors[7,25,26]. We hypothesize that antibodies that are directed against surface components further promote *S. aureus* aggregation. These large aggregates of bacteria, antibodies and other host factors in the blood may not be cleared by the host and could become trapped in various tissues (particularly in the lungs, but also in skin, the kidneys, the liver and joints) where they would fix complement, recruit granulocytes and other phagocytes and induce the release of pro-inflammatory mediators, as well as potentially causing septic emboli. Should this prove to be the case, then the expected consequences are tissue damage, ischaemia and abscess formation, which would lead to multi-organ failure and shock. Hence, in our opinion, targeting staphylococcal surface components does not seem to be a sound vaccine strategy.

Scientific evidence to support our hypothesis comes from published vaccination studies that were carried out in the Gram-positive pathogen *Enterococcus faecalis* and from recent studies with *S. aureus* in the rabbit model of infective endocarditis. It is important to consider that *S. aureus* and enterococci both form aggregates and both cause infective endocarditis. The enterococcal aggregation substance (AS), as the name suggests, promotes the formation of large bacterial aggregates and mediates attachment to eukaryotic cells[27,28]. AS is a major contributor to *E. faecalis* pathogenesis in rabbit models of infective endocarditis and sepsis[29,30]. Vaccination with heat-killed or gentamicin-treated aggregation substance-expressing (AS^+) *E. faecalis*, followed by challenge with either AS^+ or AS^- organisms, resulted in endocarditis vegetations and increased lethality in AS^+ *E. faecalis*-infected rabbits[27]. AS-specific antibodies were shown to promote the aggregation of *E. faecalis in vitro*, which suggests that antibody-mediated hyperaggregation contributes to poorer disease outcomes and increased lethality in vaccinated rabbits. However, treatment of rabbits with monovalent, AS-specific antibody fragments (IgG Fabs), which had been shown to prevent aggregation of AS^+ *E. faecalis in vitro*, effectively decreased vegetation size, reduced bacterial recovery from vegetations and increased survival when challenged with AS^+ *E. faecalis*[27].

A similar effect was recently observed with *S. aureus*[19]. Rabbits were vaccinated with an *S. aureus* multivalent surface antigen preparation that was enriched for Isds, and were challenged intravenously with a sublethal dose of *S. aureus* MW2. Vaccinated rabbits succumbed to infection within 6 hours of MW2 challenge, whereas unvaccinated rabbits, albeit sick, survived to day 4 (REF. 19). Hence, these studies suggest that *E. faecalis* and *S. aureus* share the property of increased disease severity in the presence of antibodies that target their surface components. In our opinion, the increased incidence of multi-organ failure and death in the V710-vaccinated human cohort that acquired *S. aureus* bacteraemia[4] could be explained by a similar mechanism. These effects are quite dramatic and require further investigation in *S. aureus*; however, mouse models of *S. aureus* infection do not reproduce these lethal effects.

Mice do not mimic humans

Resistance to lipopolysaccharide, cytotoxins and SAgs. Mouse immune and cardiovascular systems respond differently to bacterial toxins, compared with human and rabbit systems, which partly explains their resistance to the lethal effects of lipopolysaccharide (LPS; also known as endotoxin), SAgs and cytotoxins (TABLE 1). The lethal dose of LPS in humans is approximately 0.01 μg per kg (REF. 31), and the lethal dose in rabbits is approximately 0.5 μg per kg (REF. 32). However, in commonly used laboratory mice, the LPS lethal dose is >80,000 μg per kg (REFS 33,34). The lethal doses of cytotoxins, notably α-toxin, in humans are unknown, but α-toxin is considered to be highly lethal when administered intravenously, as evidenced in the Bundaberg, Australia, disaster in which 12 of 21 recipients of a diphtheria toxoid vaccine developed fatal disease that was associated with the production of α-toxin by an *S. aureus* contaminant[35]. In rabbits (specifically, Dutch-belted rabbits), 0.005 μg per kg of α-toxin injected intravenously kills in 24 hours and 0.5 μg per kg kills in 1 minute[36].

Box 1 | **Immunization: mouse versus human**

So far, two *Staphylococcus aureus* vaccines (StaphVAX (Nabi Biopharmaceuticals) and V710 (Merck)) and five passive immunization preparations (Pagibaximab (Biosynexus, Glaxo Smith Kline), tefibazumab (Aurexis; Inhibitex), Veronate (Inhibitex), Aurograb (Novartis) and AltaStaph (Nabi Biopharmaceuticals)) have completed clinical trials. All seven vaccines targeted *S. aureus* surface components.

Pagibaximab is a humanized monoclonal antibody that is targeted against lipoteichoic acid (LTA). LTA is an important component of the Gram-positive cell wall and is essential for its integrity and for bacterial survival[84]. Pagibaximab was tested in very low birthweight neonates for the prevention of staphylococcal sepsis[85,86]. In both studies, Pagibaximab showed a positive trend, but the results were not conclusive. A larger study was recently completed, and Pagibaximab failed to reduce staphylococcal sepsis in this population[87].

StaphVAX is a bivalent vaccine that is composed of capsular polysaccharide 5 (CP5) and CP8 individually conjugated to recombinant exoprotein A (rETA) from *Pseudomonas aeruginosa*[88]. CP5 and CP8 are highly conserved and are produced by most pathogenic *S. aureus* strains[89–92]. CP5 increased bacterial virulence in a mouse model of bacteraemia and arthritis, compared with a CP5-deficient strain[93]. Vaccination of mice with CP5–rETA provided protection against a lethal challenge[94]. In humans, StaphVAX failed to reduce the incidence of *S. aureus* bacteraemia in patients undergoing haemodialysis[95,96].

AltaStaph comprises pooled hyperimmune polyclonal antibodies from healthy individuals who have been vaccinated with StaphVAX[97]. Treatment of mice with AltaStaph significantly reduced bacteraemia and deep tissue infections after sublethal challenge[94]. Treatment of rats with rabbit CP5–rETA-specific antibodies lowered the prevalence of infective endocarditis and decreased bacterial counts in vegetations, blood and kidneys[98]. In humans, AltaStaph failed to reduce the incidence of *S. aureus* bacteraemia in low birthweight neonates[97] or to improve the outcome of patients with persistent bacteraemia[99].

Tefibazumab (which comprises humanized monoclonal antibodies) and Veronate (which is a pooled human hyperimmune preparation) target clumping factor A (ClfA). ClfA is a cell wall protein that binds to fibrinogen and fibrin and promotes bacterial clumping in plasma and adherence to blood clots. Mutational analysis provided evidence for the role of ClfA in *S. aureus* pathogenesis in a mouse model of septic arthritis and a rat model of indwelling catheter-infective endocarditis[100,101]. Vaccination of mice with recombinant truncated ClfA led to less severe arthritis after *S. aureus* challenge. In humans, tefibazumab failed to prevent *S. aureus* bacteraemia relapse, complications or death[102]; Veronate failed to reduce the incidence of late-onset sepsis in premature neonates[103].

Aurograb is a single-chain fragment of the immunoglobulin variable region that is specific for the *S. aureus* ATP-binding cassette (ABC) transporter GrfA. Human sera from 26 patients with epidemic methicillin-resistant *S. aureus* 15 (EMRSA-15) septicaemia contained high antibody titres against three different *S. aureus* ABC transporter peptides[104]. In a mouse sepsis model, treatment with human antibodies to the individual peptides significantly reduced the recovery of EMRSA-15 from the kidneys, liver and spleen[105]. Aurograb failed to show efficacy when tested as an add-on therapy in patients with severe, deep-seated staphylococcal infections[6,106].

V710 is a monovalent vaccine that is composed of the iron-responsive surface determinant B (IsdB), which is a cell wall-anchored protein that is conserved among diverse *S. aureus* strains and is expressed during iron limitation[4,107]. The vaccine provided protection against nasal carriage in a cotton rat model, increased survival in a mouse sepsis model and induced high-level antibody titres in mice[16,108,109]. In humans, V710 failed to reduce the incidence of post-operative *S. aureus* bacteraemia and major chest wound infection, and it was associated with an increased incidence of multi-organ dysfunction and increased mortality in members of the vaccinated cohort who developed *S. aureus* bacteraemia[4,110].

By contrast, in BALB/c mice, the lethal dose of α-toxin is >200,000 μg per kg (P.M.S, unpublished observations).

The difference between mice, rabbits and humans in their susceptibility to SAgs is even more striking. Humans who have been injected intravenously with SEA at doses as low as 0.001 μg per kg develop fever and hypotension (which are symptoms of TSS)[37]. In rabbits, intrapulmonary inoculation of TSST1 or continuous exposure to TSST1 at 0.05 μg per kg implanted subcutaneously in mini-osmotic pumps causes 100% lethality[24]. In mice, continuous exposure to TSST1 at 4,000,000 μg per kg in mini-osmotic pumps is not lethal[24]. To induce susceptibility to SAgs in mice, investigators have used potent sensitizing agents, such as the hepatotoxin D-galactosamine[24]; however, these mice develop what seems to be fulminant liver failure. This condition is not observed in humans or rabbits with TSS[38,39]. Therefore, standard mouse models have reduced sensitivity to LPS, cytotoxins and SAgs.

Attenuated virulence. A comparison of the virulence of representative isolates of *S. aureus* USA200, USA300 and USA400 clonal types has been carried out in animal models of pneumonia, sepsis and infective endocarditis. The USA200 isolates colonize human mucosal surfaces and are frequently associated with life-threatening infections, such as post-influenza pneumonia, surgical wound infections, infective endocarditis, sepsis and TSS[40–43]. USA200 isolates are also associated with almost all cases of menstrual TSS, which develops in otherwise healthy females[18]. USA200 strains usually produce α-toxin at low levels (0.05–5.0 μg per ml)[36,44] and TSST1 at high levels (5–20 μg per ml in liquid culture and up to 16,000 μg per ml in biofilms) or α-toxin, TSST1 and SEC, which is also produced at high levels (up to 100 μg per ml in liquid culture)[45]. In the rabbit pneumonia model, USA200 isolates cause 90% lethality in 48 hours at doses of 6.5×10^8 colony-forming units (CFUs) per kg[46]. Furthermore, in a rabbit infective endocarditis or sepsis model, USA200 isolates effectively cause lethal sepsis, with the LD_{50} (dose lethal to 50% of animals tested) calculated at $0.3–1.5 \times 10^8$ CFUs per kg (REF. 47). By contrast, in the mouse pneumonia model, USA200 strains inoculated at a dose of 1.0×10^{10} CFUs per kg fail to induce lethality in a 48 hour time period and exhibit a greatly reduced capacity to induce lethality in a sepsis model[48].

USA300 isolates are commonly community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains, which are frequently associated with skin and soft tissue infections and necrotizing pneumonia[49]. The USA300 LAC strain produces α-toxin at high levels (~150–500 μg per ml)[50] and the SAgs SE-like X (SEl-X) (~200 ng per ml in liquid culture), SEl-Q and SEl-K at low levels (<30 ng per ml)[20]. In the rabbit pneumonia model, LAC inoculated at 6.5×10^8 CFUs per kg induces 90% lethality 24 hours post-infection; whereas, in the mouse pneumonia model, 80–90% lethality is reported with an inoculum that is tenfold higher on a per-kilogram basis than that used for rabbits[51]. Furthermore, an intravenous dose of 6.5×10^8 CFUs per kg in rabbits results in 100% mortality in <18 hours, whereas a five-times higher dose is required to obtain comparable lethality (90%) in mice in 24 hours[44,52].

The USA400 strain MW2 is a CA-MRSA that was isolated from a child with lethal necrotizing pneumonia. MW2 produces moderate amounts of α-toxin (~50 μg per ml)[53] and encodes an array of SAgs,

Table 1 | **Comparison of specific characteristics of humans, mice and rabbits**

	Animal		
Property	**Human**	**Rabbit**	**Mouse**
LPS lethality	0.013 μg per kg	500*–0.5‡ μg per kg	>80,000 μg per kg
α-toxin lethality	NA	0.005 μg per kg in 24 hours	>200,000 μg per kg
Superantigen lethality	0.0013 μg per kg	50*–0.05‡ μg per kg	Not lethal at 4×10^6 μg per kg
Similarity of cardiovascular physiology to that of humans	NA	Similar to humans	Not similar to humans
Similarity of fever response to that of humans	NA	Similar to humans	Not similar to humans

LPS, lipopolysaccharide; NA, not applicable. *Young adult (2–3 kg) rabbits. ‡8 month-old rabbits.

including SEC and SEl-X. SEC is the dominant SAg in MW2 — it is produced at ~100 μg per ml in liquid culture — and is required for the development of necrotizing pneumonia and lethal sepsis in rabbits[46]. MW2 doses of 6.5×10^8 CFUs per kg are 100% lethal in rabbits 48 hours after infection. Strikingly, a tenfold increase in the MW2 dose on a per-kilogram basis results in only 20% lethality in mice and 36% lethality in rats in the same 48 hour period[54,55].

Overall, the mouse models seem to mostly reflect the effect of high levels of α-toxin production, as they completely lack sensitivity to SAgs and to lower levels of α-toxin (FIG. 2). Therefore, *S. aureus* strains such as MN8 seem to be avirulent in mice (as TSST1 has no effect and this strain produces low levels of α-toxin), MW2 seems to have greatly reduced lethality (although it produces high levels of SEC and moderate levels of α-toxin) and LAC seems to be hypervirulent (as it produces α-toxin at high levels, even though it makes SAgs at low levels). There are likely to be many additional reasons for the differential responses of mouse models compared with rabbit models and humans. However, it is clear that mouse resistance to SAgs and α-toxin produces virulence outcomes that are not consistent with those that are observed in humans and rabbits. In our opinion, the reliance on mouse models has led to confusion and has perpetuated misconceptions in the staphylococcal field of research, including our understanding of the virulence of strains relative to one another, the role of various staphylococcal proteins in disease and the identification of staphylococcal molecules that have potential as human vaccine candidates. The identification of staphylococcal molecules that have potential as vaccine candidates is the most important consequence of the reliance on mouse models, and it may have made the most important contribution to the failure of *S. aureus* vaccine development.

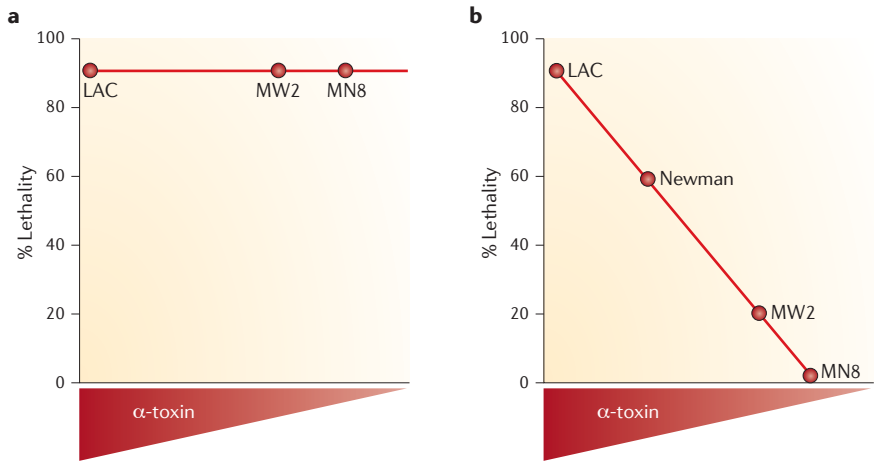

Figure 2 | **Lethality induced by various *Staphylococcus aureus* strains as a function of the animal model used (mouse versus rabbit pneumonia model) and their level of α-toxin production. a** | Inoculation of the *S. aureus* strains LAC, MW2 and MN8 at 6.5×10^8 CFUs per kg in the rabbit pneumonia model shows that they are equally virulent 48 hours after infection. **b** | Inoculation of the *S. aureus* strains LAC, Newman, MW2 and MN8 at 6.5–10 × 10^9 CFUs per kg in the mouse pneumonia model shows that virulence correlates with the level of α-toxin production. Data taken from REFS 46,48,50,51.

Basis for divergent responses

The divergent responses of mice compared with humans and rabbits result from at least three major differences. First, the mouse transcriptional response to inflammatory stimuli (such as LPS) differs considerably from that of humans. Second, the effects of cytokines on the vasculature and the central nervous system in response to LPS and SAgs are different in mice compared with humans. Third, there are differences in the composition of the gut and vaginal microbiota in mice, rabbits and humans.

Transcriptional response: endotoxemia. It has been known since the early 1960s that mice are highly resistant to the effects of Gram-negative LPS[56]. A key recent study by Seok *et al.* investigated this difference at the molecular level and showed that mouse inflammatory responses to infectious agents in general, and to LPS specifically, are significantly different from those of humans[21]. Gene expression profiling of C57BL/6J mice and humans during endotoxemia showed a low correlation between the human genes and mouse orthologues and vice versa. In this study, the human response to endotoxemia was threefold greater than that of mice and was poorly predicted by the mouse model. A comparison of the human and mouse pathways that are activated or suppressed during endotoxemia showed that humans activate Fcγ receptor-mediated phagocytosis in macrophages and monocytes, interleukin-10 (IL-10) signalling, integrin signalling, B cell receptor signalling and Toll-like receptor signalling. In mice, these signalling pathways are poorly activated, particularly the Toll-like receptor signalling pathway[21]. Most of the signalling pathways that are suppressed during endotoxemia in

humans are activated in mice, particularly the calcium-induced T cell apoptosis pathway[21]. Similar results were obtained by analysis of the genomic response of humans and mice to other severe acute inflammatory diseases, including sepsis and acute respiratory distress syndrome. This study provides evidence that, at the molecular level, mouse models poorly reflect the molecular mechanisms of human inflammatory diseases.

Cytokine response: fever and hypotension. SAgs cause fevers and life-threatening illnesses in humans, owing to overstimulation of the immune system, which leads to the massive production of IL-1β, IL-6, interferon-γ (IFNγ), tumour necrosis factor (TNF) and lymphotoxin-α[57,58] (which is commonly known as a cytokine storm). Cytokines, such as IL-1β and IL-6, are endogenous pyrogens (most of the endogenous pyrogens that cause fevers in humans are primarily produced by immune cells) and subsequently initiate a fever cascade that begins with the production of prostaglandin E2 by the hypothalamus[59,60]. It has been known for decades that BALB/c mice do not respond to LPS with fever and instead show a dose-dependent reduction in body temperature[59], whereas even minute doses of LPS cause high fevers both in humans and in rabbits[32,59]. High fevers, such as those seen in patients with TSS and a variant form of TSS known as extreme pyrexia syndrome[61], can participate in lethality.

The major pathway by which microorganisms that express LPS or SAgs cause fevers depends on interactions of these toxic molecules with immune cells via Toll-like receptors[62,63] or SAg cross-bridging of T cell receptors and major histocompatibility complex class II (MHC II) molecules[19], either peripherally or within the central nervous system[59]. As mentioned above, mice do not activate Toll-like receptor signalling in response to LPS[21]. It is also known that mouse T cells show decreased responses to SAgs, whereas both human and rabbit T cells (and antigen-presenting cells) respond comparably to SAgs[33,56,64]. The lack of susceptibility of mice to SAgs has led to attempts to humanize mice, particularly for MHC II (REFS 65–67). However, mice that are humanized for MHC II still do not respond to SAgs with all of the signs of TSS[68].

Furthermore, massive production of TNF and lymphotoxin-α has major effects on the human and rabbit cardiovascular systems, which cause capillary leak, hypotension and shock. SAgs and α-toxin (which, at high concentrations is cytotoxic but at low concentrations is highly pro-inflammatory[35]) have major roles in pneumonia, sepsis and infective endocarditis, and their effects are mediated by the induction of acute inflammation[19,50,54]. Together, these studies clearly indicate the differential production of pro-inflammatory cytokines by mice compared with rabbits and humans in response to either LPS or SAg stimulation.

Gut and vaginal microbiota. Interestingly, the susceptibility of humans and rabbits to SAgs correlates with the presence of Gram-negative bacteria that produce endotoxin shock-inducing LPS[69,70] (that is, *E. coli* in humans and *Pasteurella multocida* in rabbits) in the intestinal and vaginal tracts[71–74]. Rodents[75] and non-human primates[76] have fewer of these organisms. Young adult rabbits (~3 months old) succumb to TSST1 at doses of 50 μg per kg (REF. 39) and to LPS at 535 μg per kg (REF. 32). At 8 months old, rabbits are colonized at a greater density by *P. multocida* and become 1,000 times more susceptible to LPS (LD_{100} 0.5 μg per kg); concomitantly, the susceptibility of rabbits to the lethal effect of TSST1 increases 1,000-fold (LD_{100} 0.05 μg per kg). Enhancement of LPS shock by TSST1 was shown in young adult rabbits that were administered TSST1 at increasing concentrations before LPS treatment[32]. TSST1 increases the susceptibility of rabbits to LPS lethality by up to 10^6-fold[32]; this effect has been observed with all SAgs that have been tested[32,77]. The SAg-induced increase in LPS lethality results from impaired LPS clearance by the liver[78], possibly via direct liver cytotoxicity and the synergistic effects of SAgs and LPS on the production of TNF[33]. Endotoxemia occurs in humans during TSS and in rabbits that have been injected with TSST1. This has led to the hypothesis that animals with a gut and vaginal microbiota that includes Gram-negative bacteria expressing LPS that is capable of inducing endotoxin shock are susceptible to SAgs plus LPS[79,80], whereas animals such as rodents and non-human primates, in which Gram-negative bacteria that express toxic LPS are rarely detected, are resistant[76,81]. Although synergism between TSST1 and LPS does occur in mice and rhesus macaques that have been injected with the toxins, the susceptibility to the SAg–LPS combination never reaches that of rabbits[33]. However, it supports the idea that the resistance or susceptibility of mice and monkeys versus rabbits and humans results not only from differences in cardiovascular physiology but also from the presence of endogenous LPS and combined toxin effects on the immune system. On this basis, one would expect that mice could predict disease in non-human primates, whereas rabbits predict disease in humans.

Vaccination is possible

Targeting secreted virulence factors. Vaccination with purified SAgs or SAg toxoids alone or in combination with cytotoxins has proven to be a successful strategy to protect rabbits against lethal doses of USA100, USA200, USA300 and USA400 *S. aureus* clonal types and provides sterilizing immunity[82]. Vaccination with TSST1, SEC or SEB protects rabbits from lethal challenge with CA-MRSA $TSST1^+$ USA200, SEC^+ USA400 or SEB^+ USA400 strains, respectively[46]. Three different TSST1 toxoids produce TSST1-neutralizing antibodies at high levels and protect rabbits from a TSST1 lethal challenge in an LPS enhancement model. A trivalent vaccine that is composed of $TSST1_{G31S}$ (or $TSST1_{S32P}$), SEC and α-$toxin_{H35L}$ provides complete protection against a lethal challenge with *S. aureus* MNPE ($TSST1^+$, SEC^+ and α-$toxin^{HI}$) in the rabbit pneumonia model[47]. Furthermore, a pentavalent vaccine that contains $TSST1_{G31S}$ (or $TSST1_{S32P}$), SEC, α-$toxin_{H35L}$, β-toxin and γ-toxin protects rabbits from infective endocarditis and lethal sepsis when challenged with a lethal dose of *S. aureus* MNPE[47]. Studies in rabbits thus provide evidence that a multivalent vaccine targeting secreted staphylococcal virulence factors that are produced by nearly all *S. aureus* isolates protects against life-threatening staphylococcal diseases[82]. These studies suggest that the neutralization of crucial SAgs and cytolysins effectively incapacitates *S. aureus* such that phagocytic elimination of the organism occurs, similarly to what would be expected for coagulase-negative staphylococci (coagulase-negative staphylococci produce fewer virulence factors than other *S. aureus* strains and are eliminated by innate immune mechanisms, such as the alternative complement cascade and neutrophil opsonization).

Conclusions

The virulence of many bacterial pathogens that cause major human diseases is often multifactorial. The diseases that these organisms cause are becoming increasingly difficult to treat and require new thinking in vaccination strategies. So far, researchers have typically used rodents — particularly mouse models — to study human diseases that are caused by these bacterial pathogens, not because mouse infections mimic human illnesses, but rather owing to costs, the

availability of genetically defined mutants and pressure from granting and regulatory agencies and reviewers. It is noteworthy that the pressure from granting agencies is beginning to change; for example, the US National Institutes of Health (NIH) recently issued a programme announcement to develop alternative animal models[83].

Understanding *S. aureus* pathogenesis is crucial for the development of new treatment and vaccine strategies. In our opinion, success in these areas will rely on researchers being willing to switch to using animal models with immune systems and cardiovascular physiologies that more closely resemble those of humans, as such models are more likely to be predictive of human disease. This switch must take place if the biomedical research community is to develop effective vaccines and immunotherapeutic agents to target microorganisms that are increasingly difficult to treat.

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Competing interests statement
The authors declare no competing interests.