Published in final edited form as:
Expert Opin Ther Targets. 2012 June ; 16(6): 601–612. doi:10.1517/14728222.2012.682573.

The potential use of toxin antibodies as a strategy for controlling acute *Staphylococcus aureus* infections

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Abstract

Introduction—The pandemic human pathogen, *Staphylococcus aureus*, displays high levels of antibiotic resistance and is a major cause of hospital- and community-associated infections. *S. aureus* disease manifestation is to a great extent due to the production of a large arsenal of virulence factors, which include a series of secreted toxins. Antibodies to *S. aureus* toxins are found in people who are infected or asymptomatically colonized with *S. aureus*. Immunotherapies consisting of neutralizing anti-toxin antibodies could provide immediate aid to patients with impaired immune systems or in advanced stages of disease.

Areas covered—Important *S. aureus* toxins, their roles in pathogenesis, rationales for selecting *S. aureus* toxins for immunization efforts, and caveats associated with monoclonal antibody-based passive immunization are discussed. This review will focus on hyper-virulent community-associated methicillin-resistant *S. aureus* (CA-MRSA) because of their recent surge and clinical importance.

Expert opinion—Antibodies against genome-encoded toxins may be more broadly applicable than those directed against toxins found only in a sub-population of *S. aureus* isolates. Furthermore, there is substantial functional redundancy among *S. aureus* toxins. Thus, an optimal anti-*S. aureus* formulation may consist of multiple antibodies directed against a series of key *S. aureus* genome-encoded toxins.

Keywords

Toxin; vaccine; immunotherapy; methicillin-resistant *Staphylococcus aureus*; monoclonal antibody; alpha-hemolysin; phenol-soluble modulins; superantigens; leukotoxins; Panton-Valentine leukocidin

1. The need for immunotherapies and vaccines against *Staphylococcus aureus*

The commensal microorganism, *Staphylococcus aureus*, is a primary colonizer of human nares (in ~ 25% of the population) and the etiological agent of many diseases [1, 2]. Since the

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Declaration of Interest:
This work was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.

1960s, the use of antibiotics to treat *S. aureus*-associated infections has become increasingly problematic owing to the emergence of methicillin-resistant *S. aureus* (MRSA) and *S. aureus* strains that are resistant to a wide variety of different antibiotics [2, 3]. Originally a pathogen primarily of immune-compromised and otherwise predisposed patients in hospital settings (hospital associated-MRSA; HA-MRSA) [4], more aggressive methicillin-resistant strains of *S. aureus* have recently appeared in the community (community-associated-MRSA; CA-MRSA), which may successfully infect healthy human people [1–3]. The majority of CA-MRSA infections are soft skin and tissue infections (SSTIs) (~ 50% of total cases). However, invasive cases of CA-MRSA-related diseases, such as necrotizing pneumonia (~ 5% of total cases), are becoming increasingly more widespread. As a consequence, the treatment of MRSA-infections places great financial strains on public healthcare [5].

It is commonly believed that the epidemiological success of the pulsed-field type USA300 CA-MRSA isolate, which is now pandemic in the United States, is due to a combination of high virulence, antibiotic resistance, and colonization capacity, leading to sustainable spread in the community [6]. The high number of community-associated infections with USA300 has prompted considerable research efforts aimed to develop therapeutics to combat CA-MRSA. These primarily include strategies interfering with virulence, including most notably active and passive immunization efforts directed against CA-MRSA toxins and other virulence factors (reviewed extensively in [7–10]).

Here, an overview on a number of *S. aureus* toxin families, with a focus on *S. aureus* toxins encoded exclusively by, or showing increased expression in, CA-MRSA strains will be provided. The roles that those toxins have in *S. aureus* pathogenesis and disease will be discussed. Finally, the rationales and caveats regarding the use of anti-toxin monoclonal antibodies (mAbs) as therapeutics for the prevention and treatment of CA-MRSA-mediated disease will be explored.

2. Toxins involved in *S. aureus* pathogenesis

S. aureus produces and secretes many types of toxins with diverse roles in pathogenesis, particularly affecting immune evasion and activation of the immune response. These include alpha-hemolysin (α-hemolysin, Hla), beta-toxin (β-toxin), the superantigens (SAgs), the leukotoxins, and the phenol-soluble modulins (PSMs). The genes coding for β-toxin, the SAgs and leukotoxins are mostly encoded by mobile genetic elements (MGEs) [11]; therefore, toxin expression can differ tremendously between different strains of *S. aureus*. Only a few *S. aureus* toxins, such as α-hemolysin and the PSMs, are genome-encoded [12, 13] and expressed by both HA- and CA-MRSA strains. However, α-hemolysin and the PSMs are expressed more strongly in CA-MRSA compared to HA-MRSA strains. This suggests that differences in gene regulation may influence the epidemiological success of CA-MRSA strains such as USA300 [14, 15]. For example, it has been noted that USA300 shows increased expression of the global regulatory quorum sensing system, accessory gene regulator (*agr*) [16], which controls the expression of many virulence factors in a cell density-dependent manner. The *agr* locus consists of a divergent promoter, which controls the transcription of RNAII and RNAIII. The RNAII transcript contains 4 genes encoding a classical two-component quorum sensing system (*agrAC*), an auto-inducing peptide (AIP) precursor (*agrD*), and the AIP maturation and exporter protein (*agrB*). Upon increasing concentration of AIP during growth, the AgrAC two-component system becomes activated at a certain threshold concentration of AIP. Upon binding of AIP to the membrane protein AgrC, AgrA is activated, which in turn binds to the divergent promoter and induces transcription of RNAII and RNAIII. RNAIII is a regulatory RNA molecule that regulates the expression of a majority of toxins [16]. *S. aureus* mutants lacking a functional *agr* regulatory

system are significantly less virulent than the corresponding wild-type strains, which has recently been demonstrated specifically for USA300 [14, 17–19].

2.1 Leukotoxins

Neutrophils, or polymorphonuclear leukocytes (PMNs), are key components of the innate immune system and involved in controlling *S. aureus* infection [20, 21]. Possibly for that reason, *S. aureus* produces a large variety of virulence factors that inhibit neutrophil function [22], allowing *S. aureus* to circumvent elimination by innate host defense [23]. Particularly, pore-forming, bi-component leukotoxins with cytolytic affinity towards cells of myeloid lineage, such as monocytes, macrophages and neutrophils, represent key contributors to *S. aureus* immune evasion [24, 25]. Each leukotoxin requires one class S and one class F protein, which are individually non-toxic, to form a β-barreled pore-forming structure upon oligomerization [26]. Six class S subunits (LukS-PV, HlgA, HlgC, LukE, LukM, LukH) and five class F subunits (LukF-PV, HlgB, LukD, LukF′-PV, LukG) have been described [24, 25, 27, 28]. One exception to this monogamous pairing is the γ-hemolysin gene cluster, which comprises three genes ((*hlgA* [or *hlg2*], *hlgC* [or *lukS*] and *hlgB* [or *lukF*]), whose gene products allow the formation of two functional pairs of proteins: Hlg [HlgA + HlgB] and Luk [HlgB + HlgC]. Notably, all leukotoxins reportedly contribute to disease progression in at least some animal infection models, with the exception of LukM/LukF′-PV [29–31].

While the *hlg* gene cluster occurs in 99% of *S. aureus* strains [32–34], many other leukotoxin genes are not uniformly present among *S. aureus* isolates. The *lukDE* [35] and *lukGH* (*lukAB*) [24, 25] genes can be found in many sequenced strains or clinical isolates including USA300 [31]. However, for example, *lukDE* are not found in the HA-MRSA isolate, USA200 [36] and the phage-encoded *lukF-PV* and *lukS-PV* [33], which code for Panton-Valentine leukocidin (PVL), are mostly restricted to CA-MRSA strains [37], represented by only ~ 5% of all clinical isolates. Of note, the role of PVL in *S. aureus* pathogenesis remains controversial despite extensive research that has been performed on that leukotoxin [1, 15, 38, 39]. Owing to the initially observed epidemiological link of *lukSF-PV* with CA-MRSA, it has been suggested that PVL contributes to the pathogenesis of typical CA-MRSA disease manifestations, such as necrotizing pneumonia [15, 40] or the formation of skin abscesses [41, 42]. However, PVL-negative CA-MRSA strains have since been isolated, indicating that this epidemiological correlation is not absolute [1, 15]. Furthermore, animal infection studies investigating the role of PVL in *S. aureus* disease are not in agreement [43–50]. These discrepancies may in part be due to differences of the animal models used, such as inoculum size and host species. Over the last decade, the PVL controversy may have overshadowed the importance of other, and perhaps more relevant, leukotoxins that are expressed by a majority of CA-MRSA strains, such as *lukDE* and *lukGH*. These leukotoxins are now being investigated more intensely. Studies using isogenic *S. aureus* deletion mutants show that LukDE and LukGH contribute to the virulence of *S. aureus* in murine sepsis [36] and renal abscess models [25], respectively. Furthermore, there have been some, albeit relatively few studies on the contribution of γ-hemolysin to *S. aureus* pathogenesis and disease. These studies indicate that γ-hemolysin may have roles in septic arthritis and weight loss in mice [51] and endophthalmitis in rabbits [52]. However, it is unknown whether LukDE, LukGH or γ-hemolysin contribute towards other facets of HA- and CA-MRSA disease, for example, severe cases of pneumonia, as has been suggested for PVL.

2.2. Staphylococcal superantigens

The superantigen (SAg) family consists of small, serologically distinct proteins including staphylococcal enterotoxins (SEs) A–E, G–J, staphylococcal enterotoxin-like toxins (SEl)

K-R, U and X, and Toxic Shock Syndrome Toxin-1 (TSST-1), all of which are ~ 20 – 28 kDa in size [34, 53, 54]. Members of the SAg family are responsible for food poisoning, toxic shock syndrome and respiratory disease [55]. The SAgs share a common protein structure [53, 56] that enables each SAg to crosslink different alleles of major histocompatibility complex (MHC) class II molecules on the surfaces of antigen-presenting cells with different alleles of the variable region of T-cell receptors (TCRs) [57–59]. These interactions allow the SAgs to bypass the conventional antigen processing pathway to non-specifically activate T-cells (~ 50%) and induce the irregular production of high levels of inflammatory cytokines [60]. This event induces multisystem disease that includes rash, hypotension, pyrexia, emesis and diarrhea [61]. The SAgs also cause severe life-threatening multiple organ failure [61].

Of the large number of SAgs that have been described, only the recently discovered SElX is genome-encoded [54]; all other SAgs are encoded by MGEs [62–64]. Moreover, not all *S. aureus* isolates display superantigenic activity [65] because the SAgs are not distributed equally among *S. aureus* strains [66]. Furthermore, it is not unusual to see varied levels of SAg production among *S. aureus* strains that express SAgs [67–72]. This is attributed to the involvement of at least three global regulators; *agr* [73], *sarA* [74], σ^B and *saeRS* [75]. Interestingly, TSST-1 and SEB have been reported to act as negative global regulators of toxin production [76].

The roles of SAgs in the pathogenesis of *S. aureus* disease have been investigated in animal models by administration of purified SAgs [77]. Other studies have compared the differences in the capacities of naturally occurring SAg^+ and SAg^- strains [78] and isogenic SAg mutants [54, 79–81] to cause disease in animals. The overall importance of the SAgs in CA-MRSA pathogenesis is debatable, because many SAgs that are classically associated with *S. aureus* disease are not expressed by CA-MRSA strains. For instance, USA300 does not typically produce TSST-1, SEB or SEC, SAgs that are most often associated with toxic shock [73, 82]. As one noticeable exception, SElX has been shown to contribute to USA300 necrotizing pneumonia in rabbits [54]. However, it is unknown if SElX contributes to other manifestations of CA-MRSA disease, such as SSTIs.

2.3. Alpha-hemolysin

The genome-encoded α-hemolysin (Hla), one of the first toxins described for *S. aureus*, has recently received considerable attention as a target for an anti-*S. aureus* vaccine and the production of neutralizing antibodies for passive immunization. α-hemolysin is produced by a majority of *S. aureus* strains and its expression is regulated by at least three global regulatory systems, which include *agr* [83]. It is well known for its pore-forming [13, 84–89] and pro-inflammatory [90] properties. However, its cognate receptor, the zinc-dependent metalloprotease ADAM10 (A disintegrin and metalloproteinase 10), was only recently discovered [91, 92]. α-hemolysin contributes significantly to the pathogenesis of *S. aureus* USA300-induced skin infection and pneumonia [46, 93, 94]. Mice that do not express ADAM10 are resistant to lethal infection with *S. aureus* strain USA300 in a pneumonia model [91], but it is not known if ADAM10 is also required for α-hemolysin induced abscess formation by *S. aureus*. Another signal, which is found on immune cells, may also be required for α-hemolysin-induced pneumonia by *S. aureus* [95]. This second signal belongs to a class of cytosolic proteins called nucleotide-binding domain and leucine-rich repeat containing (NLR) proteins, which are important activators of the innate immune response. NLRs defend against pathogen infection and endogenous damage in response to a variety of immunogenic stimuli [96]. Upon activation, several members of this family assemble into large multimeric protein complexes called inflammasomes, which induce inflammatory responses. NLRP3 in particular is activated in response to bacterial toxins [97], including *S. aureus* β-toxin, γ-hemolysin [98] and α-hemolysin [95]. Thus, NLPR3 inflammasome

activation and the presence of ADAM10 appear to contribute to the development of *S. aureus* pneumonia.

2.4 Phenol-soluble modulins

The phenol-soluble modulins (PSMs) are a family of small, amphipathic peptides with cytolytic and pro-inflammatory properties towards a range of cells types including erythrocytes and neutrophils [12]. The pro-inflammatory properties of PSMs (such as, induction of intracellular calcium flux, chemotaxis, and cytokine production in neutrophils) are dependent on binding to their cognate receptor, human formyl peptide receptor 2 (FPR2). Of note, PSM-dependent cell lysis occurs independently of FPR2 binding [99]. Two PSM sub-families can be distinguished: PSMα peptides, which include the well-known δ-toxin, are 20 – 26 amino acids, and PSMβ peptides 43 – 44 amino acids in length [12]. Generally, members of the PSMα class show more pronounced pro-inflammatory and especially lytic activities than members of the PSMβ class [12].

Similar to many other *S. aureus* virulence determinants, PSMs are under control of the *agr* global regulatory system [12, 100]. However, in contrast to most Agr targets, they are regulated directly by AgrA rather than via RNAIII [100, 101]. Most *S. aureus* strains contain the RNAIII-embedded *hld* gene encoding δ-toxin [102], and the operons coding for the *psmα* and the *psmβ* genes, which are located on two separate loci [12]. In contrast to the ubiquitous prevalence of the *psmα* and the *psmβ* genes, the *psm-mec* gene [103] is restricted to HA-MRSA strains carrying staphylococcal cassette chromosome *mec* (SCC*mec*) types II, III and VIII [101].

Importantly, CA-MRSA isolates secrete higher amounts of PSMs in average compared to HA-MRSA isolates [12], suggesting that the PSMs have a prominent role in CA-MRSA pathogenesis. Indeed, isogenic CA-MRSA *psmα* mutants are severely impaired in their ability to form abscesses and cause sepsis in mice [12]. Collectively, the α-class PSMs, including δ-toxin, and the β-class PSMs all play a role in the dissemination of CA-MRSA from biofilm-related catheter infections in mice [104]. Biofilms are sticky agglomerations consisting of a mixture of bacterial cells and extracellular polysaccharide matrices and are intrinsically resistant to antibiotics and mechanisms of host defense [105]. The detergent-like quality of PSMs [12, 106] influences the development of mature biofilms by breaking interactions between cell populations, introducing channel formation, followed by the detachment of cell clusters, leading to bacterial dissemination in vivo [104, 107]. Finally, PSMs act synergistically with β-toxin [108, 109] and PVL [110] in vitro. Since important CA-MRSA isolates do not produce β-toxin [111] and not all CA-MRSA isolates harbor the *lukF-PV* and *lukS-PV* genes, this synergism may infer a cooperative relationship between virulence factors produced by different strains of *S. aureus* that have different toxin expression profiles.

3. Anti-toxin antibody responses in humans

Soon after birth, 40 – 50% of the population carry *S. aureus*. By 14 months of age, there can be a ~ 40% reduction in carriage rates [112, 113] and *S. aureus* can remain a persistent colonizer in the nares of adults in ~ 25% of the population [114]. In healthy human individuals, antibody responses toward many *S. aureus* antigens can be detected, such as cell surface components, non-protein antigens, and toxins [54, 115–118], indicating that *S. aureus* antigens are immunogenic.

Antibodies against most *S. aureus* toxins are not readily detected in healthy newborns although anti-TSST-1 antibodies were reported [119]. In healthy adults, carriers of *S. aureus* can produce greater levels of antibodies towards *S. aureus* antigens than non-carriers, but

these titers are generally less pronounced at the age of 65 and higher [116]. Of the anti-toxin antibodies, only significantly higher levels of anti-TSST-1 and anti-SEA have been described in carriers compared to non-carriers; and it is thought that previous exposure(s) to different *S. aureus* strains may account for the diverse levels of antibody titers between individuals [116, 117].

Individuals infected with *S. aureus* generally produce greater levels of antibodies to PVL, α-hemolysin, and SAgs than healthy individuals [44, 115, 119, 120]. Furthermore, low levels of antibodies may be associated with more severe *S. aureus* infections [120]. However, presence of antibodies is not necessarily associated with protection, as recently described for the role of anti-PVL antibodies in PVL+ CA-MRSA infections in humans [121].

Sera from *S. aureus*-infected patients may demonstrate neutralizing activity against *S. aureus* toxins, such as SAgs [117, 122, 123] but whether there is neutralizing activity of these sera towards other *S. aureus* toxins remains to be determined. Altogether, although much information has been garnered from these serological studies, the roles that anti-*S. aureus* antibodies play during infection still remains largely unknown.

4. *S. aureus* toxins as targets for monoclonal antibody therapy

Unlike active immunization, which sometimes requires repeated boosters and long periods of time for maximum immune responses to be generated, passive immunization would provide immediate treatment for unvaccinated patients to help reduce the severity of acute *S. aureus* disease. Perhaps even more importantly, mAb therapy would benefit immune-compromised patients or infants with immature immune systems or even add to the existing pool of potentially protective anti-*S. aureus* antibodies. Clearly, it is important for such therapy to take effect that *S. aureus* is rapidly identified. Moreover, late stages of *S. aureus* infection may not be amenable to passive therapy, as advanced stages of toxin-mediated tissue destruction will have ensued. Even though there are caveats to passive immunization, much research has recently been performed on neutralizing mAbs against *S. aureus* toxins; and some of these successfully protected from *S. aureus* infection in animal models (Table 1). Polyclonal anti-toxin antibodies also are protective in animal models of *S. aureus* infection (Table 1). However, many of the investigated toxins, such as PVL and SEB, are not expressed by all *S. aureus* strains. Therefore, anti-PVL and anti-SEB antibodies may only be useful in treating acute infections caused by PVL and SEB-expressing *S. aureus* strains. Instead, core-genome encoded toxins, such as α-hemolysin, SElX, LukGH, and PSMs may better serve as targets for antibody generation, as antibodies against those toxins may be more broadly applicable for acute cases of *S. aureus* infections. The use of these toxins as targets for mAb generation will be discussed in more detail below.

4.1 Antibody therapy against the leukotoxins

Even though there is controversy over whether PVL is a decisive virulence determinant in *S. aureus*, there is still strong interest in creating a vaccine against PVL and producing anti-PVL mAbs. There are several points supporting the use of anti-PVL antibodies for passive immunization. First, humans are capable of generating humoral immune responses against PVL [115, 117, 121]. Second, polyclonal and mAbs to PVL are neutralizing [121, 124, 125]. Third, injection of PVL together with a tetravalent anti-PVL mAb, which binds both LukS-PV and LukF-PV antigens simultaneously, reduced inflammation and tissue destruction in a non-lethal model of endophthalmitis in rabbits [124]. Finally, anti-PVL mAbs exhibit cross-reactivity with other leukotoxins, such as HlgC [124]. On the other hand, there are many important findings that negate the benefits of using anti-PVL antibodies. First, polyclonal anti-PVL antibodies failed to neutralize the cytotoxicity of a PVL-expressing USA300 strain toward human neutrophils in vitro [126]. Second, anti-PVL immune sera did not protect from

S. aureus pneumonia in passive immunization experiments using mice [46]. Third, PVL-neutralizing antibodies did not protect children from primary or recurrent CA-MRSA-associated SSTIs [121]. Finally, the presence of anti-PVL antibodies may in fact exacerbate disease caused by PVL-expressing CA-MRSA during early stages of infection [127]. These antibodies may prevent the interactions of PVL with cells of the innate immune system, resulting in poor activation of the immune response and a decreased ability to mount an attack to the infection.

LukDE and LukGH (LukAB) also interact with innate immune cells [24, 25] and are lytic toward neutrophils [24, 25]. Furthermore, PVL and LukGH lyse other innate immune cells, such as human monocytes and macrophages [25, 128]. *S. aureus* strains that express one or more of these leukotoxins at sufficient levels thus may severely impair the ability of the host to mount an effective immune response. However, it remains to be investigated whether antibodies to leukotoxins other than PVL also may enhance the virulence of *S. aureus* in a way similar to anti-PVL antibodies. Finally, it has been reported that active immunization with PVL subunits relieves mice of severe skin and intranasal infection with PVL-expressing CA-MRSA [44]. Phase II clinical trials are currently underway using a recombinant LukS-PV toxin subunit. The results of this study have yet to be released [129].

Taken together, there are strong arguments against the use of anti-PVL antibodies to treat CA-MRSA disease. Furthermore, there is currently no compelling evidence for a necessary role of PVL in CA-MRSA pathogenesis, leaving the possibility that CA-MRSA strains without PVL may further expand or PVL-positive CA-MRSA strains may lose the PVL-encoding prophage without a substantial loss of virulence characteristics. Moreover, pursuing development of an anti-PVL mAb- or antigen-based vaccine is problematic from a marketing point of view, because only ~ 5% of *S. aureus* clinical isolates possess the genes coding for PVL, limiting its applicability. Finally, although active immunization with PVL subunits appears to be an encouraging alternative to passive immunization, active vaccination with a combination of LukGH or LukDE subunits may help protect against a larger number of *S. aureus* infections.

4.2 Antibody therapy against the superantigens

The SAgs are responsible for a range of symptoms and diseases in humans that range from food poisoning to toxic shock. Passive therapy with anti-SAg antibodies would likely help alleviate the symptoms associated with SAg-related disease by preventing the interactions of the SAgs with their ligands. Antibodies against TSST-1, SEA, SEB, SEC are readily detected in healthy individuals [117, 130–132] and individuals with active *S. aureus* infections [119], indicating that these SAgs are immunogenic.

There is particular interest in developing neutralizing antibodies against the category B bioterrorism agent, SEB, which is resistant to denaturing and highly toxic to humans [133, 134]. Anti-SEB mAbs are neutralizing in vitro [135] and passive therapy with anti-SEB antibodies successfully prevents SEB-induced disease manifestations in mice [136] and monkeys [137] against lethal challenge with purified SEB. While there is certainly cross-reactivity of anti-SAg mAbs with several SAgs [138], it is unlikely that a monovalent SEB mAb will protect from infection by many SEB^- expressing *S. aureus* strains of high clinical importance, such as the pandemic CA-MRSA strains. In contrast, passive immunization with several different anti-SAg mAbs would potentially be able to protect against a larger variety of *S. aureus* strains. Furthermore, the genome-encoded SElX may serve as an attractive alternative target for generating mAbs, because it is expressed by a majority of *S. aureus* isolates including the CA-MRSA strain USA300 [54]. Finally, passive immunization is not the only strategy for interfering with SAg-producing strains; other strategies include active immunization with detoxified SAgs [139–141], the use of soluble forms of engineered

TCR Vβ proteins [142], and hybrid TCR Vβ/MHCII proteins [143] that can block the binding activity of SEB toward its natural ligands found on T cells and antigen-presenting cells.

4.3 Antibody therapy against α-hemolysin

Healthy human individuals that are colonized with *S. aureus* produce high levels of antibodies directed toward α-hemolysin [116, 117]. As α-hemolysin is expressed by a majority of *S. aureus* strains, passive immunization with mAbs to α-hemolysin may be effective against many different *S. aureus* strains including strains of CA-MRSA. Several studies have shown the protective efficacy of anti-α-hemolysin antibodies in *S. aureus* abscess [144, 145] and pneumonia [46, 146] murine infection models. In addition, active immunization with a detoxified α-hemolysin derivative (H35L) was shown to reduce the severity of *S. aureus*-induced pneumonia [46] and *S. aureus* abscess [145] infections. Interestingly, recombinant α-hemolysin toxoid is being tested in the same phase II clinical trial as recombinant LukS-PV [129].

4.4 Antibody therapy against the phenol-soluble modulins

The genome-encoded PSMs show little variation in amino acid sequences between *S. aureus* strains [12]. Therefore, antibodies against PSMs could serve as an effective passive immunization strategy against a broad spectrum of *S. aureus* strains. Infants with invasive *S. aureus* infections do not appear to produce antibodies to the strongly cytolytic PSMα3 [147], but anti-PSM antibodies can be raised in animals injected with PSMs and adjuvant [107, 148] indicating that the PSMs have immunogenic potential. High levels of PSMs are produced by CA-MRSA strains in vitro [12, 14], but the levels of PSM in-vivo production are yet unknown. Therefore, the lack of anti-PSM antibody production, which is observed at least in infants, may either be due to a low production level of PSMs under those conditions, or if the infection is localized, such as in a SSTI, the host may not be able to detect enough PSMs in order to generate antibodies. Antibodies against PSMs may be more readily detected in adults or in other types of *S. aureus* diseases. However, this needs to be determined by including the relatively recently discovered PSMs as antigens in future human serological studies.

Although *S. aureus* catheter-related biofilm infections are not a focus of this review, this subject area warrants a brief discussion. PSMs play a crucial role in biofilm structuring and dissemination of biofilm-associated infection in these organisms [104, 107]. Notably, anti-PSMβ antibodies blocked the dissemination of *S. epidermidis* catheter infection in mice. Therefore, passive immunization with anti-PSM antibodies may be useful to inhibit the dissemination of bacteria from biofilm formed on catheters. However, more research is clearly needed to investigate antibody therapy against PSMs.

5. Expert opinion

There is an urgent need for research aimed to find a successful immunization strategy against *S. aureus* infections. This is warranted in particular because of the high levels of antibiotic resistance shown by pandemic strains of *S. aureus*. Antibodies against *S. aureus* toxins may have particular value, as they neutralize the binding affinity of the toxins, preventing pro-inflammatory responses and cytolytic activities. Furthermore, they may block toxin oligomerization even after insertion into cell membranes, thus preventing cytolytic activity of the toxin.

Choosing a specific *S. aureus* toxin as a singular vaccine target is complicated by the fact that *S. aureus* produces many, often functionally redundant toxins. Some toxins are immunogenic and play important roles in *S. aureus* pathogenesis, but many are not expressed by all *S. aureus* isolates. Therefore, targeting core-genome encoded toxins, such

as α-hemolysin, LukGH, SElX or the PSMs, would serve as a better basis for vaccine development than toxins only produced by a sub-population of strains, such as PVL, TSST-1, SEA, SEB and SEC, especially when aiming for a vaccine preparation with broad applicability.

Monoclonal antibodies raised against only one *S. aureus* antigen are protective in animal models of *S. aureus* infection, but frequently, only disappointing results were achieved with many of those mAbs in human phase 1/2 clinical trials (reviewed in [149]). An alternative, and perhaps more successful, strategy would comprise a mAb preparation consisting of antibodies against different toxins and possibly other *S. aureus* virulence factors, such as surface proteins, to simultaneously target establishment and exacerbation of infection. In theory, such antibody therapy would thus be able to inhibit different aspects of *S. aureus* pathogenesis. Interestingly, it was recently shown that administration of two different mAbs against *S. aureus* SEB led to significantly more protection than one singular SEB-directed mAb [136]. This concept is not new - similar results have been achieved with mAbs against toxins produced by other bacterial species, such as *Bacillus anthracis* anthrax toxin [150], *Clostridium* botulinum neurotoxin A [151] and *C. difficile* toxins A and B [152]. However, it needs to be further explored whether such a vaccination strategy, i.e. using antibodies against different *S. aureus* antigens, would be broadly effective against *S. aureus*. Moreover, it needs to be cautiously evaluated whether antitoxin antibodies may have the opposite effect of exacerbating *S. aureus* disease, such as in the case of passive immunization with anti-PVL antibodies [127].

In summary, unsuccessful clinical trials with predominantly non-toxin-based *S. aureus* antigens warrant new approaches to protect against the new wave of pandemic CA-MRSA strains in addition to the continuously high disease burden due to HA-MRSA. There is strong evidence from laboratory experiments suggesting that passive immunization approaches using anti-toxin antibodies may be as successful as those directed toward other *S. aureus* antigens. However, human clinical trials will always be required as ultimate proof of efficacy against *S. aureus* infection [9, 136, 153].

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Highlights

- Passive immunization with one antibody or active immunization with one antigen may not be sufficient to protect against different epidemic *S. aureus* strains.
- Genome-encoded virulence factors, such as SElX, PSMs and α-hemolysin, as targets would allow universal treatment against different epidemic *S. aureus* strains.
- Active immunization against leukotoxins may be advantageous. However, other leukotoxins, such as LukDE and LukGH, should be considered in addition to PVL.
- Passive immunization with multiple mAbs against different *S. aureus* virulence factors may be more beneficial than passive immunization with one mAb.

Table 1

Validated toxin targets

Toxin	Challenge agent	Antibody type	Model	Species	Observed effect	Reference
α-hemolysin	*S. aureus* USA300	Rabbit polyclonal	Pneumonia	Mouse	+	[46]
	S. aureus USA300	Mouse monoclonal	Abscess	Mouse	+	[146]
	S. aureus USA300, CC30, CC5	Mouse humanized monoclonal	Abscess	Mouse	+	[144]
	S. aureus USA300	Rabbit polyclonal	Abscess	Mouse	+	[145]
SEB[a]	SEB	Mouse monoclonal	SEB-induced lethal shock	Mouse	+	[136]
	SEB	Chicken polyclonal	Aerosolized SEB	Monkey	+	[137]
PVL[b]	PVL	Mouse humanized heavy chain	Endophthlamitis	Rabbit	+	[124]
	S. aureus USA300 PVL+	Rabbit Polyclonal	Pneumonia	Mouse	−	[46]
	Several *S. aureus* USA300 PVL+ strains	Rabbit Polyclonal	Abscess	Mouse	−	[127]
***S. epidermidis* PSMβ**[c]	*S. epidermidis* 1457	Mouse polyclonal	Subcutaneous catheter-associated biofilm model	Mouse	+	[107]

Abbreviations;

[a]Staphylococcal enterotoxin B,

[b]Panton-Valentine leukocidin,

[c]Phenol-soluble modulin,