Strengthening the immune system as an antimicrobial strategy against *Staphylococcus aureus* infections

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Staphylococcus aureus asymptomatically colonises the nose of about 20% of the healthy population, but can also cause mild to severe infections. Antibiotic treatment of these infections is not always successful, resulting in substantial therapy failure. Therefore, more effective treatment is urgently required. Among alternative antimicrobial intervention strategies, both active and passive immunisation in the prevention and cure of *S. aureus* infection is investigated in experimental animals and patients. The translational value of animal studies is determined by a proper selection of bacterial target, infection model, treatment modalities, and outcome parameters. In experimental animals, various infection models are described for studying the efficacy of immunisation. Most of these studies focussing on a broad range of bacterial targets were successful in prevention, reduction or cure of infection. The efficacy of immunisation focused on a limited number of bacterial targets was also investigated in *S. aureus* infected patients or individuals that are at high risk for *S. aureus* infection. In these studies, final conclusions on the efficacy of immunisation cannot be drawn.

Keywords *Staphylococcus aureus*; immunotherapy; animal models; clinical trials

1. Introduction

About 20% of the healthy population persistently carries *S. aureus* in their nose [1]. Carriage of *S. aureus* is asymptomatic, although it can also cause infection. *S. aureus* infection represents a serious burden to global public health, particularly with respect to infections in healthcare-related settings. In this respect, *S. aureus* is not only associated with serious infections such as surgical wound infections and pneumonia, but also with serious invasive disease, including sepsis, which may result in endocarditis, osteomyelitis and meningitis [2]. Further, MRSA has now become prevalent within hospitals, leading to a significantly increased risk of mortality and increased length of stay for hospitalised patients [3]. Alarmingly, recent years have also witnessed the spread of resistant staphylococci outside the healthcare setting, with the prevalence of community-acquired MRSA now increasing [4]. Even more alarming is the emergence of vancomycin- and mupirocin-resistant *S. aureus* strains, which represent a major threat to the control of *S. aureus* infections within both the hospital and community setting [5,6]. These worrying developments indicate that there is an urgent need to develop novel long-lasting anti-staphylococcal therapies to enhance, or even replace, current antibiotic-related therapies. Among alternative, non-antibiotic-related, antimicrobial intervention strategies, both active and passive immunisation in the prevention and cure of *S. aureus* infection is investigated in experimental animals and patients. These novel treatment strategies need to be thoroughly evaluated before they can be applied within the clinical setting. Although *in vitro* assays provide a hint of the efficacy of both active and passive immunisation strategies against *S. aureus* infection, they may be inadequate or even misleading for the prediction of actual *in vivo* efficacy. Therefore, studies *in vivo* are of high importance. When utilizing *in vivo* animal models, a well-planned experimental study design is essential in order to generate data with a high translational value for patients using a minimum input of animals. In this respect, a proper selection of the bacterial target, infection model, treatment modalities, and outcome parameters is crucial. In this chapter, an overview of the study design as well as the results obtained with immunisation in experimental animals and clinical studies is provided.

2. Bacterial target

Selection of the *S. aureus* target is the first and probably most essential step in designing a study on immunisation as antimicrobial strategy. The criteria to which the ideal target should meet need to be defined.

Immunogenicity of the bacterial target is required to be successful in immunisation studies in *S. aureus* infection. This means that antibodies against the target are expected to be produced. An indication which *S. aureus* antigen should be targeted with immunisation is provided by studies comparing anti-staphylococcal antibody levels in *S. aureus* carriers or *S. aureus* infected patients with non-carriers or non-infected patients, respectively. Antibodies against these antigens may play important roles in *S. aureus* colonisation and infection. For example, IgG against SEA and TSST-1 is elevated in carriers compared to non-carriers [7], while IgG against glucosaminidase, HlgB, LukF-PV, SA0688, SCIN, SSL1, and SSL5 are higher in patients with *S. aureus* bacteraemia than in non-infected patients [8].

Although a broad panel of *S. aureus* antigens appears to be immunogenic, these antibodies are not always sufficient to eliminate *S. aureus* from the nose of *S. aureus* carriers [7]. Moreover, the anti-staphylococcal antibodies present in healthy individuals do not prevent them from getting infected with *S. aureus* as carriers of *S. aureus* have higher anti-

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staphylococcal antibody levels [7] and also a higher chance on developing *S. aureus* infections than non-carriers [9]. This might support the exclusion of these antigens as target for immunisation as antimicrobial strategy.

Immunisation based on more than one *S. aureus* antigen as target is expected to be more valuable than immunisation focusing on a single antigen, as each *S. aureus* carrier or *S. aureus* infected patient develops a unique immune response against different *S. aureus* antigens. Another reason for the selection of multiple targets for immunisation strategies is the presence of functional redundancy. *S. aureus* produces many antigens, of which some have similar or comparable functions. When one of these antigens is absent in a *S. aureus* strain, another antigen will take over this function.

S. aureus targets without sequence variation in the immunoglobulin binding domain should be preferred above targets with genetic variation. This increases the chance that the variable region of the antibodies, either induced by active immunisation or administered as passive immunisation, is able to bind to the epitope on the *S. aureus* target, and will enhance a successful treatment of the *S. aureus* infection. As McCarthy and Lindsay [10] showed sequence variation in 25 surface bound *S. aureus* proteins as well as in 13 secreted proteins amongst the 58 *S. aureus* genomes studied, they emphasise that immunisation as antimicrobial strategy should contain (antibodies against) cocktails of antigens representing all variants of the *S. aureus* targets.

A polyvalent strategy may not be necessary when the *S. aureus* target is conserved on all *S. aureus* strains. Choosing a conserved *S. aureus* target will enhance the success rate of immunisation as antimicrobial strategy in infections which are caused by a wide variety of *S. aureus* strains. This is of main importance as the *S. aureus* strains causing infections in patients are very heterogenous [8,11]. Ziebandt *et al* [11] observed that 7 extracellular proteins were produced by all 17 clonally different *S. aureus* strains (IsaA, Lip, LytM, Nuc, SA0620, SA2097, and SA2437). A further 9 proteins (Aur, Geh, GlpQ, α-toxin, HlgB, SA0570, SA1812, SspA and SspB) were identified in at least 80% of these strains. Especially the 7 proteins are interesting targets for monovalant immunisation strategies towards all *S. aureus* strains.

Another consideration that has to be taken into account when selecting the bacterial target for immunisation as antimicrobial strategy is that the selected target needs to be accessible for antibodies enabling binding of antibodies to the target. To this aim, the target needs to be exposed on the bacterial surface, or has to be excreted. In addition, target expression during *S. aureus* infection is needed.

3. Treatment modalities

Important choices concerning treatment modalities are active or passive immunisation as well as dosing.

3.1. Active or passive immunisation

In active immunisation, also called vaccination, antigenic material (vaccine) is administered before infection, and aims for stimulation of the immune system to develop adaptive immunity. This vaccine may consist of intact, but inactivated or attenuated *S. aureus* cells, or purified components of the bacterium. In case the target is immunogenic, active immunisation will result in an antibody response directed against the target. Moreover, the antigen will elicit an effector T cell response.

The use of an adjuvant in active immunisation is highly preferred in order to induce inflammation at the injection site, which will enhance the immune response. It is known that immunisation with purified proteins without adjuvant leads to a poor immune response. In addition, adjuvants cause soluble protein antigens to aggregate and precipitate forming particles, which will facilitate their efficient uptake by antigen-presenting cells and reduce the rate at which the antigen is cleared from the system. Various adjuvants are used in active immunisation studies concerning *S. aureus*. CFA is commonly used. This is an emulsion of killed mycobacteria and mineral oil into which antigens are vigorously mixed. In experimental animals, the use of CFA should be limited because of its painful reaction and potential for tissue damage. IFA only contains the mineral oil, and is often used as adjuvant in booster immunisations after CFA as adjuvant in the first immunisation. Next to CFA/IFA, Ribi adjuvant, AAHSA, cholera toxin, toxoids, saponin, Abisco-100 and alum are also described as adjuvants in active immunisation against *S. aureus* infections in experimental animals.

In passive immunisation, antibodies directed against the bacterial target are injected into the body. Passive immunisation can be administered before infection (prophylactic treatment) or after infection (therapeutic treatment). Although passive immunisation will provide a quick response, the benefit is short-lasting as the antibodies once injected will naturally be broken down. In passive immunisation, the type of antibodies used is of importance. Antibodies can be monoclonal or polyclonal, directed against one or more epitopes of the target, respectively. Next, antibodies may be derived from human origin in order to be directly applicable in the clinical setting, or they may have an animal origin, with better chances for protective efficacy in an animal model.

3.2. Dosing

All kinds of dosing schedules and routes of administration are used in the various studies on active and passive immunisation as antimicrobial strategy.

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In studies concerning active immunisation in experimental models, animals are immunised at least two times, and in one study even ten times. Booster immunisations after the primary exposure may lead to long-lasting immunity through activation of immunological memory cells. Time intervals between two consecutive injections vary as well. In most studies, this interval is 1-2 weeks. Immunisation injections are mostly given via the s.c. or i.m. route, while i.n., i.p., i.v., and i.t. injections are also described. In some studies, two routes of administration are combined. In general, dose-finding is necessary for each target and route of administration. In contrast to the animal studies, in clinical studies active immunisation is performed only once, via the i.m. route.

In passive immunisation studies in most experimental models, animals are immunised with a single prophylactic gift of antibodies. This gift is administered 2 days to several hours before infection. In only a few passive immunisation studies, therapeutic treatment is applied. The antibodies are mainly administered via the i.p. route, while i.v. administration is also described. Again, dose-finding is necessary. In contrast to the animal studies, in clinical studies passive immunisation is performed via the i.v. route, either as prophylactic or as therapeutic treatment. Number of immunisations range from a single gift to immunisation twice daily.

4. Outcome parameters

To monitor the course of infection and to assess the efficacy of immunisation, relevant outcome parameters are of high importance.

Overall, in experimental animal studies, clinical signs of illness, including body temperature, behaviour, and appearance are monitored over time. In models of skin infection, also the lesion size over time is monitored. In lethal infection models, disease progression score over time is assessed. In all models, after dissection of the animals, bacterial load in blood and relevant organs may be determined. It always needs to be confirmed that the *S. aureus* isolate recovered from the infected animals is identical to the *S. aureus* isolate inoculated.

In clinical studies, next to clinical signs of illness, prevention of (relapse of) *S. aureus* infection is an important outcome parameter to assess the efficacy of immunisation as antimicrobial strategy.

5. Experimental animal studies

Regarding the design of *in vivo* studies in experimental animals and the interpretation of the results obtained, one has to realise that animal models of infection only mimic the infection in humans, mainly because the infective dose used in artificially induced infections in animals is different from the naturally acquired infection in patients. At the same time, animal models – provided well characterised – are needed and accepted as well for studying the potential efficacy of novel treatment strategies, as they provide the unique opportunity to study treatment efficacy under similar conditions of intensity and duration of infection. Uniform groups of patients are difficult to obtain because of differences in underlying clinical conditions.

A proper experimental design when performing animal studies is always required to generate maximum results with a minimum input of animals. Next to choice of bacterial targets, treatment modalities and outcome parameters, proper selection of the infection model is of main importance.

5.1. Infection model

The experimental infection model is characterised by the animal species, type of infection, and the infecting *S. aureus* strain used. Relevant choices have to be made in light of the research question that needs to be answered, and will be discussed.

5.1.1. Animal species

In experimental models of *S. aureus* infection, a variety of animal species are used. A prerequisite of the selected animal species is that it has to be susceptible to *S. aureus*.

Mice are often used as experimental animals, being relatively inexpensive compared to other animal species, easy to maintain and easy to handle. Moreover, much is known about mouse genetics and immunology, and knock-out mouse strains are available. In addition, immunologic reagents applicable in mouse tissues are widely available.

In experimental *S. aureus* studies, next to different strains of mice, also rats, cotton rats, rabbits, sheep, cattle and rhesus macaques are used for evaluation of the efficacy of active or passive immunisation. The cotton rat has been described as a good model for *S. aureus* nasal colonisation as nasal histology of cotton rats is comparable to that of humans. Moreover, pretreatment with antibiotics, being required to establish nasal colonisation in mice, is not needed in cotton rats [12]. In contrast to rodents and rabbits, sheep and cattle may be naturally colonised and infected by *S. aureus*. This is interesting for studying the potential efficacy of treatment. However, these *S. aureus* strains are genetically different from those found in humans [13]. Rhesus macaques, being naturally colonised by *S. aureus* in their

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noses are the most suitable experimental animals [14]. However, while rhesus macaques are genetically more related to humans than cotton rats, sheep and cattle are, ethical issues limit the use of large groups of macaques.

In addition to the selection of the animal species, the microbial status of the experimental animal needs attention. Microbiological standardisation is an important tool to achieve reproducible animal experiments. Most commercial suppliers of experimental animals have a health monitoring program based on the FELASA Recommendations [15]. The supplier lists the organisms that are not present in the experimental animal. In this respect, animals with a SPF status are not tested for the presence of *S. aureus*, while those with a SOPF status are free of *S. aureus*. In order to maintain the microbial health status in the facility were the animal experiments are performed, it is important that animals are housed in individually ventilated cages. Disinfected gloves should be worn when handling these animals, to prevent transmission of human colonising *S. aureus* strains to the animals.

5.1.2. Infection type

S. aureus can cause a variety of infections [9], ranging from mild skin infections to more severe infections like arthritis, endocarditis and sepsis, as shown in Fig. 1. Many types of infection models are described, some more extensively characterised than others. For each research question, the most suitable experimental infection should be selected, and clinically relevant infections that urgently need alternative treatment should be preferred. The following models have been published for studying immunisation as antimicrobial strategy in *S. aureus* infections:

- nasal carriage
- skin infection, wound infection
- keratitis
- mastitis
- catheter-related infection
- endocarditis
- renal abscess
- pneumonia
- septic arthritis
- bacteraemia
- sepsis
- shock

5.1.3. Infecting strain

Many different *S. aureus* strains were used among which clinical isolates as well as sequenced strains. Originally, sequenced strains were clinical isolates as well, but they are passaged *in vitro* for many times. The choice of *S. aureus* strain depends on the research question.

As bacterial strains may lose virulence by *in vitro* passage, animal passage of the infecting strains is needed to maintain virulence of the *S. aureus* strain.

6. Results of immunisation in experimental infection models

Numerous studies in animal models are published concerning immunisation against *S. aureus* infections, including a wide range of bacterial targets and both active and passive immunisation. Most studies concentrate on a specific infection model, and the experimental approach in these studies is summarised in Table 1.

6.1. Nasal carriage

In this infection model, mice or cotton rats are i.n. challenged with *S. aureus*. To assess the efficacy of immunisation, noses are surgically removed and homogenised to determine numbers of viable *S. aureus*.

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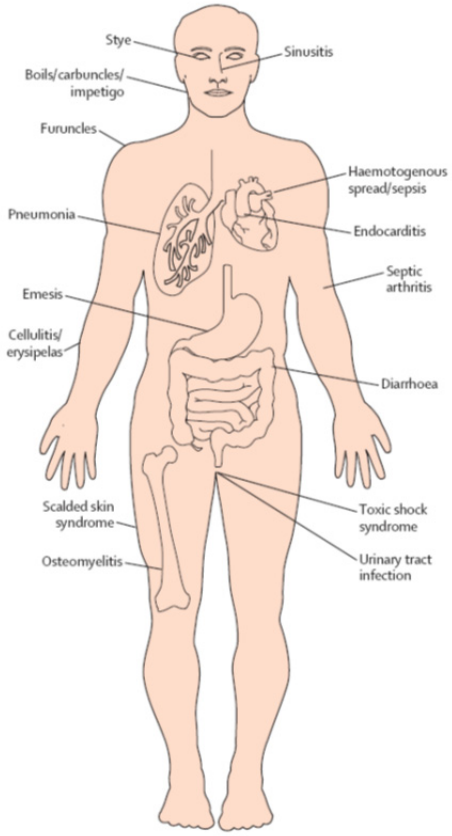

Fig. 1 *S. aureus* can cause a wide range of infections. Figure adapted from Wertheim *et al* [9].

Active immunisation with IsdA, IsdH, FmtB, SA2666 or the glucosaminidase domain of Atl was studied by Clarke *et al* [16]. Only immunisation with IsdA or IsdH protected against nasal carriage.

Schaffer *et al* [17] investigated whether active immunisation with ClfB resulted in lower *S. aureus* load in the nose of mice. While s.c. immunisation with ClfB resulted in lower *S. aureus* colonisation levels, i.n. immunisation with ClfB did not protect mice against nasal colonisation. Passive immunisation targeting both CP5 and ClfB reduced the bacterial load in the nose of mice as well, as observed by Pozzi *et al* [18].

6.2. Skin infection, wound infection

For the model of skin infection, mice are challenged with *S. aureus* via the i.d. or s.c. route. In some cases, bacteria are mixed with dextran beads to promote abscess formation. Efficacy parameters include body weight changes, size of the abscess, hyperaemia (increased blood flow), skin injury, and viable *S. aureus* load in the abscess.

Kennedy *et al* [19] studied both active and passive immunisation focused on α-toxin in a murine model of skin infection. Both immunisation strategies resulted in reduction of abscess size. Passive immunisation targeting α-toxin was also studied by Foletti *et al* [20]. They also observed a reduced abscess size and a reduction of the bacterial burden in the abscess. Active immunisation with LukF-PV or LukS-PV was studied by Brown *et al* [21]. While s.c. immunisation protected mice against body weight loss, i.n. immunisation was not effective.

Three studies used *S. aureus* mixed with dextran beads for infection. Park *et al* [22] infected mice with *S. aureus*, and at the same time, mice were passively immunised targeting AIP-4. This passive immunisation reduced hyperaemia and skin injury. In the study of Balaban *et al* [23], mice were actively immunised with RAP. This immunisation reduced the incidence of cutaneous lesions, as well as the lesion size. Pozzi *et al* [18] passively immunised mice targeting PNAG and α-toxin; CP5 and ClfB; or CP8 and IsdB. These immunisations resulted in a reduction in bacterial load in the abscesses.

A model of wound infection in mice was described by Schennings *et al* [24]. A full thickness wound was punched out through the skin, which was subsequently infected with *S. aureus*. Mice were actively immunised with Eap, ClfA, and Efb. This reduced the number of infected wounds.

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Table 1 Experimental approach in animal studies on immunisation as antimicrobial strategy in *S. aureus* infection.

Experimental model	Reference	Experimental animal	Bacterial target	Active immunisation			Passive immunisation			
				Number of immunisations	Route of immunisation	Adjuvant	Number of immunisations	Route of immunisation	P/T[1]	Type of antibodies[2]
Nasal colonisation	[16]	Cotton rat	IsdA,IsdH,FmtB,SA2666,Atl	3	s.c.	Ribi				
	[17]	Mouse	ClfB	1 or 2	s.c.	CFA, IFA				
				3	i.n.	Cholera toxin				
	[18]	Mouse	CP5, ClfB				1	i.p.	P	Rabbit pAb
Skin infection	[19,20]	Mouse	α-toxin	3	i.m.	CFA, IFA	1 or 2	i.p.	P	Rabbit, human pAb
	[21]	Mouse	LukF-PV, LukS-PV	2	s.c.	CFA, IFA				
				5	i.n.	Cholera toxin				
	[22]	Mouse	AIP-4				1	i.p.	P	Mouse mAb
	[23]	Mouse	RAP	1	?	CFA				
	[18]	Mouse	PNAG, α-toxin, CP5, ClfB, CP8, IsdB				2	i.p.	P	Rabbit pAb
Wound infection	[24]	Mouse	Eap, ClfA, Efb	3	s.c.	Abisco-100				
Keratitis	[25]	Rabbit	α-toxin	3	s.c.	CFA	1	in cornea	P	Rabbit pAb
Mastitis	[26]	Rabbit	α-toxin	8	i.m., i.v.	CFA, -				
	[27]	Cattle	SEC	3	i.m.	-				
	[28,29, 30]	Cattle, sheep	Whole cell	2 or 3	s.c.	α- and β-toxoids, IFA	1	i.p.	P	Rabbit pAb
	[31,32]	Mouse	FnbpA	2	s.c.	CFA, IFA	1	intra-mammary	P	Rabbit pAb
	[33]	Mouse	ClfA				1	i.p.	P	Human mAb
Catheter-related infection	[34,35]	Mouse, rat	IsdB				1	i.p.	P	Mouse, human mAb
	[36]	Mouse	IsaA				2	i.v.	P	Mouse mAb
Endocarditis	[37]	Rat	Fnbp	3	s.c.	CFA, IFA				
			Collagen binding protein	3	s.c.	CFA, IFA				
	[38,39]	Rabbit	ClfA				1	i.v.	P/T	IGIV with elevated anti-ClfA levels
	[40,41]	Rat	CP5, teichoic acid	3 or 9	s.c.	-	1	i.p.	P	Rabbit pAb
	[42]	Rabbit	Whole cell	10	i.v., i.p.	-, -				
	[43]	Rat	FnbpB	3	s.c.	Saponin				
Renal abscess	[44,45]	Mouse	IsdA, IsdB, SdrD, SdrE	2	i.m.	CFA, IFA	1	i.p.	P	Rabbit pAb
	[46]	Mouse	Protein A	2	i.m.	CFA, IFA				
	[47]	Mouse	PNAG	3	i.p.	-	2	i.p.	P	Rabbit pAb
			CP5+CP8				2	i.p.	P	Human pAb

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Table 1 Experimental approach in animal studies on immunisation as antimicrobial strategy in *S. aureus* infection (continued).

Experimental model	Reference	Experimental animal	Bacterial target	Active immunisation			Passive immunisation			
				Number of immunisations	Route of immunisation	Adjuvant	Number of immunisations	Route of immunisation	P/T[1]	Type of antibodies[2]
Pneumonia	[21,48]	Mouse	α-toxin	2	i.m.	CFA, IFA	1	i.p.	P	Rabbit pAb
			LukF-PV, LukS-PV	2	s.c.	CFA, IFA	1	i.p.	P	Rabbit pAb
				5	i.n.	Cholera toxin				
	[18,20,49]	Mouse	α-toxin, PNAG	2	i.m.	CFA, IFA	1 or 2	i.p.	P/T	Mouse, rabbit, human mAb
Septic arthritis	[50]	Mouse	ClfA	2	s.c.	CFA, IFA	1	i.p.	P	Rat, rabbit, human pAb
	[51]	Mouse	Cna	3	s.c.	CFA, IFA				
Bacteraemia	[43,52]	Mouse	FbnpA, FnbpB				1 or 2	i.v.	P	Rat, rabbit pAb
	[53]	Mouse	ABC transporter				1	i.v.	T	Rabbit pAb
	[54]	Mouse	CP5, CP8				1	s.c.	P	Human pAb
Sepsis	[45]	Mouse	IsdA, IsdB, SdrD, SdrE	2	i.m.	CFA, IFA				
	[34,35,44,55,56,57]	Mouse	IsdA, IsdB	3	i.m.	AAHSA	1	i.p.	P	Mouse, human mAb, rabbit pAb
	[51,58]	Mouse	Cna, Fnbp	3	s.c.	CFA, IFA, -	1	i.p.	P	Rat pAb
	[59]	Mouse	SEA	1 or 3	s.c.	CFA, IFA	1	i.p.	P	Rat pAb
	[60]	Mouse	SEC	3	i.n.	Cholera toxin				
	[54]	Mouse	CP5	3	s.c.	Exoprotein A of *P. aeruginosa*				
	[61]	Mouse	Hypothetical protein 2160	4	i.p., s.c.	CFA, IFA				
	[20,22,62]	Mouse	ClfA, AIP-4, α-toxin				1	i.p.	P	Mouse, human mAb
	[47,63,64]	Mouse	PNAG				1 or 2	i.p.	P	Rabbit pAb, human mAb
	[36]	Mouse	IsaA				2	i.v.	P	Mouse mAb
	[65]	Mouse	Whole cell				2	i.v., i.p.	P	IGIV
Shock	[66,67]	Rhesus macaque	SEB	3	i.m., i.t.	Alum	?	?	P/T	Chicken pAb

[1] Prophylactic (P) or therapeutic (T) treatment.
[2] Polyclonal antibodies (pAb), monoclonal antibodies (mAb).

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6.3. Keratitis

One study, by Hume *et al* [25], investigated both active and passive immunisation targeting α-toxin in a keratitis model. In this infection model, rabbits are infected with *S. aureus* in the cornea. Both immunisation strategies targeting α-toxin protected against cornea erosion.

6.4. Mastitis

Mice, rabbits, sheep or cattle are challenged with *S. aureus* in their mammary glands. Prevention of mastitis is the parameter to assess efficacy of immunisation.

In the study of Adlam *et al* [26], rabbits were actively immunised with α-toxin, resulting in prevention of mastitis.

Active immunisation with SEC was studied by Chang *et al* [27] in a mastitis model in cattle. Immunisation prevented mastitis in lactating cattle.

Active immunisation of cattle with whole, inactivated *S. aureus* was investigated by Nordhaug *et al* [28], resulting in a reduced incidence of spontaneous *S. aureus* mastitis. Leitner *et al* [29] actively immunised cows with killed *S. aureus* strains, and showed that reduction of the number of infected cows was obtained.

FnbpA was the target for both active and passive immunisation of mice in the studies of Mamo *et al* [31,32]. Both ways of immunisation prevented mastitis.

Tuchscherr *et al* [33] passively immunised mice targeting ClfA, CP5, or CP8, resulting in reduction of the *S. aureus* load in the infected mammary glands.

Watson [30] used a mastitis model in sheep. In this study, animals were actively immunised with killed cells of *S. aureus*, which resulted in a decline of mastitis.

6.5. Catheter-related infection

For the catheter-related infection model, mice or rats are used. A central venous catheter is surgically implanted. After this surgery, *S. aureus* is inoculated i.v. or via the catheter. Bacterial load on the catheter and in blood is determined to assess the efficacy of immunisation. In one study also the *S. aureus* load in liver, lung, spleen and kidneys is used as outcome parameter.

Passive immunisation targeting IsdB was studied by both Brown *et al* [34] and Ebert *et al* [35], in models in mice and rats. Brown *et al* observed a higher number of culture-negative catheters in mice. In the study of Ebert *et al*, passive immunisation targeting IsdB was successful as well: a reduction of bacterial load in blood and on the catheter was observed in rats.

In a study of Lorenz *et al* [36], IsaA was the target for passive immunisation in their model in mice. This resulted in a lower bacterial load in the kidneys, while the *S. aureus* load on the catheter was not reduced.

6.6. Endocarditis

In the endocarditis model, rats or rabbits are used. A catheter is introduced via the right carotid artery into the left ventricle, touching the aortic valve. *S. aureus* is inoculated via the i.v. or i.p. route. Efficacy parameters include the staphylococcal load on the heart valves and the attached vegetations, in blood and in kidneys.

Schennings *et al* [37] studied active immunisation of rats with Fnbp or collagen binding protein. While active immunisation with Fnbp reduced the bacterial load on heart valves and attached vegetations, immunisation with collagen binding protein did not.

Vernachio *et al* studied the efficacy of IGIV from plasma donors with elevated levels of anti-ClfA antibody. In their first study [38], rabbits were treated with this IGIV in addition to vancomycin treatment. This resulted in a reduction of bacteraemia. When in a second study [39], this IGIV was tested alone in the same infection model, a lower bacterial load on cardiac valve vegetations and in blood was obtained.

CP5 was the target selected for immunisation in studies by Lee *et al* [40] and Nemeth and Lee [41] in rats. Lee *et al* studied passive immunisation, while in the study of Nemeth and Lee, rats were either actively immunised with killed *S. aureus* cells expressing CP5, or passively immunised. Whereas Lee *et al* observed a lower prevalence of endocarditis in immunised rats [40], this was not observed in the study by Nemeth and Lee [41]. Next to passive immunisation targeting CP5, Nemeth and Lee also studied passive immunisation targeting teichoid acid. This strategy did not protect against staphylococcal endocarditis.

Active immunisation of rabbits with killed whole cell *S. aureus* was studied by Greenberg *et al* [42]. This did not result in reduction of the incidence of endocarditis or renal abscesses.

Rennermalm *et al* [43] published a variation on the catheter-related infection model: a combined arthritis/endocarditis model. In this model, rats are i.v. challenged with *S. aureus*, and a corticosteroid is injected into the mandibular joint resulting in septic arthritis in the joint. After a week, animals are catheterised to induce endocarditis. Animals were actively immunised with FnbpB, which did not protect rats against the development of arthritis, but reduced the bacterial load on aortic valves as well as in the kidneys.

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6.7. Renal abscess

Mice are used for the renal abscess model. *S. aureus* is inoculated i.v., and to assess the efficacy of immunisation, the kidneys are removed to determine the bacterial load and to study histopathology.

Stranger-Jones *et al* [45] actively immunised mice with IsdA, IsdB, SdrD and/or SdrE. While immunisation with individual antigens did not protect against *S. aureus* challenge, the combined vaccine reduced the bacterial load in kidneys below detection level, and prevented the formation of abscesses in the kidneys.

Kim *et al* [44] studied IsdA and IsdB as targets for active immunisation in mice. Passive immunisation reduced the bacterial load in the kidneys as well as the number of abscesses. The size of abscesses was not reduced.

The group of Kim also performed a study on active immunisation with protein A in mice [46]. They observed a reduction in bacterial load in the kidneys, as well as smaller and less abscesses in this organ.

PNAG was the selected target in a study of McKenney *et al* [47]. Both active and passive immunisation strategies in mice resulted in a reduction of staphylococcal load in the kidneys. In the same study, passive immunisation targeting CP5 and CP8 was examined, which did not result in a reduced bacterial load in the kidneys.

6.8. Pneumonia

In this infection model, mice are challenged i.n. by *S. aureus*. Disease progression and animal survival, staphylococcal load in lungs and pathology of the lungs are parameters to assess the efficacy of immunisation strategies.

In a number of studies, immunisation focused on α-toxin was investigated. Bubeck Wardenburg *et al* [48] observed in mice that active immunisation resulted in a reduced mortality, as well as a reduced number of *S. aureus* in lungs and less pathology. Passive immunisation of mice showed similar results. Ragle *et al* [49] performed an almost identical study in mice. Results obtained were identical to those of Bubeck Wardenburg *et al*. Foletti *et al* [20] included only passive immunisation targeting α-toxin in their study, which completely protected mice against death. In a study of Pozzi *et al* [18], passive immunisation targeting both PNAG and α-toxin resulted in 100% survival of mice as well.

LukF-PV and LukS-PV were targets selected for active immunisation by Brown *et al* [21]. While i.n. immunisation protected mice against death, s.c. immunisation did not. Remarkably, passive immunisation targeting LukF-PV and LukS-PV, used by Bubeck Wardenburg *et al* [48], did not protect mice against pneumonia.

6.9. Septic arthritis

In the model of septic arthritis, mice are challenged i.v. with *S. aureus* and develop septic arthritis when untreated. Bacterial load in joints, degree of arthritis (assessed by clinical evaluation and histopathology) and animal survival are evaluated to assess the efficacy of immunisation.

A study by Josefsson *et al* [50] examined whether active and passive immunisation targeting ClfA protected mice against septic arthritis. Active immunisation with ClfA resulted in less severe arthritis. Passive immunisation targeting ClfA suppressed the development of arthritis and protected mice against septic death.

Nilsson *et al* [51] evaluated active immunisation with Cna, which resulted in less severe arthritis and improved the survival of mice.

6.10. Bacteraemia

Mice are infected with *S. aureus* via the i.v. or i.p. route, resulting in bacteraemia. The parameters used to assess the efficacy of immunisation include body weight and the *S. aureus* load in blood, spleen, liver, kidneys and peritoneal cavity.

Passive immunisation targeting FnbpB was studied by Rennermalm *et al* [43]. Immunisation resulted in less decrease of body weight. Rozalska and Wadström [52] passively immunised mice targeting FnbpA or FnbpB, which resulted in a reduction of the bacterial load in liver and peritoneal cavity, and a more rapid clearance of bacteria from blood.

An ABC transporter was target for passive immunisation in the study of Burnie *et al* [53]. This resulted in a lower bacterial load in spleen, liver and kidneys.

Passive immunisation targeting CP5 and CP8 was studied by Fattom *et al* [54]. This resulted in less bacteraemic mice.

6.11. Sepsis

In this model, mice are challenged with *S. aureus* via the i.p. or i.v. route, resulting in sepsis. Disease progression is scored, and parameter for effective immunisation is improved survival of animals.

Many targets for immunisation were studied in the sepsis model in mice. Stranger-Jones *et al* [45] assessed the efficacy of active immunisation with IsdA, IsdB, SdrD and/or SdrE. Remarkably, only the combination vaccine protected mice against death, while immunisation with individual antigens did not.

In contrast to these observations, Kuklin *et al* [55] and Joshi *et al* [56] showed that active immunisation with IsdB alone resulted in improved animal survival. Passive immunisation targeting IsdB improved survival of mice as well

[34,35,56,57]. Kim *et al* [44] passively immunised mice targeting IsdA or IsdB, which also protected against septic death.

Zhou *et al* [58] studied active immunisation with both Cna and Fnbp. This resulted in improved survival of mice. Nilsson *et al* [51] studied passive immunisation targeting Cna. This also protected mice against death.

SEA was selected by Nilsson *et al* [59] as target for active and passive immunisation. Both immunisation strategies improved survival of mice. The target selected for active immunisation in the study of Hu *et al* [60] was SEC. Immunisation improved animal survival.

Active immunisation with CP5 was studied by Fattom *et al* [54]. This resulted in improved mouse survival.

Glowalla *et al* [61] used hypothetical protein 2160 for active immunisation. This strategy improved the survival of mice.

A number of different research groups showed that passive immunisation targeting ClfA, PNAG, AIP-4, IsaA, or α-toxin protected mice against septic death [20,22,36,47,62-64].

Commercially available human IGIV was used by Farag *et al* [65]. IGIV administered via i.v. route did not protect mice against septic death, while i.p. administration of IGIV improved animal survival.

6.12. Shock

In contrast to the infection models mentioned above, in this model, rhesus macaques are used. These animals are not challenged with viable *S. aureus*, but with an aerosol of a lethal dose of SEB. Disease progression (anorexia, progressive depression, shock) and animal survival are outcome parameters to assess the efficacy of immunisation.

Lowell *et al* [66] actively immunised macaques with SEB. This resulted in protection against severe illness and death. Passive immunisation targeting SEB was studied by LeClaire *et al* [67]. These antibodies protected macaques against death.

7. Clinical studies

While most immunisation strategies in experimental animals have been proven to be effective in prevention, reduction or cure of *S. aureus* infection, clinical trials in humans are performed to investigate this treatment strategy. In contrast to studies in animals, it is always difficult to obtain uniform groups of patients in clinical trials due to variation in underlying diseases and in history of exposure to *S. aureus*. Proper definition of the patient population is of major importance.

Regarding the immunisation studies against *S. aureus* infection, in all patients groups *S. aureus* infections are highly prevalent.

8. Results of immunisation in clinical Phase I and Phase II/III studies

A limited number of bacterial targets for immunisation were studied in humans. In Phase I studies, safety, tolerability and immunogenicity are tested in healthy adults. In Phase II/III studies, safety, tolerability, immunogenicity and sometimes efficacy are studied in *S. aureus* infected patients or individuals that are at high risk for *S. aureus* infection. The results obtained are summarised in this way.

8.1. Phase I studies

For SA75®, a vaccine composed of whole killed *S. aureus*, a Phase I study is finished. SA75® was shown to be safe and immunogenic [68]. Currently, no further studies with this vaccine are running.

PentaStaph® is a vaccine comprising CP5, CP8, polysaccharide type 336, PVL, and α-toxin, studied in Phase I [69]. However, this product is currently abandoned.

Three other Phase I studies are ongoing. One study concerns the safety and immunogenicity of GSK Biologicals Staphylococcal 4-component Investigational Vaccine (GSK2392102A). Two studies assess the safety of a 4-antigen *S. aureus* vaccine (SA4Ag) or a 3-antigen *S. aureus* vaccine (SA3Ag). In these two studies, the effect on the presence of *S. aureus* on the skin and within the nose, throat and perineum of healthy adults is determined as well [70].

8.2. Phase II/III studies

A number of Phase II/III clinical studies in *S. aureus* patients or individuals at high risk for *S. aureus* infection are summarised below.

8.2.1. Patients receiving haemodialysis

As *S. aureus* infection is a prominent cause of complications and death among patients receiving haemodialysis [71], adequate treatment to prevent these infections is needed.

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StaphVAX® is a vaccine containing CP5 and CP8. These polysaccharides are conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A. The vaccine was administered by a single i.m. injection. This active immunisation did not provide protection against *S. aureus* bacteraemia. In a post-hoc analysis, evaluating the performance of the vaccine during various shorter time periods, StaphVAX® was shown to reduce *S. aureus* bacteraemia through 40 weeks follow-up [72,73].

Another vaccine, containing whole killed *S. aureus* combined with α-toxoid, was also investigated for prevention of staphylococcal infection in patients receiving haemodialysis. However, no effects of active immunisation were found on the incidence of peritonitis, catheter-related infections, or *S. aureus* nasal carriage [74].

8.2.2. Patients undergoing cardiothoracic surgery

Patients undergoing cardiothoracic surgery are also a group of patients that needs adequate treatment, as infections due to *S. aureus* are serious complications, resulting in substantial morbidity and mortality [75].

V710® is a vaccine containing IsdB. This vaccine was administered once via the i.m. route to patients undergoing cardiothoracic surgery. The rate of all surgical site or invasive *S. aureus* infections was not reduced. This active immunisation strategy was associated with even higher mortality among patients who developed *S. aureus* infection [76].

Currently, studies with the vaccine StaphVAX® (described in 8.2.1) concerning safety and immunogenicity in patients undergoing cardiothoracic surgery are running [70].

8.2.3. Patients undergoing orthopaedic joint surgery

In orthopaedic patients, *S. aureus* accounts for the majority of surgical site infections [77]. A treatment strategy that provides protection during the post-operative period would address an important unmet medical need.

Safety and immunogenicity of the vaccine StaphVAX® (described in 8.2.1) are currently assessed in patients undergoing orthopaedic joint surgery [70].

8.2.4. Patients with bacteraemia

S. aureus is a leading cause of both hospital- and community-acquired *S. aureus* bacteraemia, and the current therapy is often not successful [78]. More powerful treatment is needed.

Aurexis® (tefibazumab) is a humanised monoclonal antibody that binds to ClfA. In patients with positive *S. aureus* blood cultures, who were treated with standard antibiotic therapy, this passive immunisation was administered once via the i.v. route, which was well tolerated. However, the number of patients developing *S. aureus* bacteraemia-related complications that were not present at baseline or developing relapse of bacteraemia was equal in patients treated with Aurexis® compared to those who received placebo-treatment [79].

AltaStaph® contains polyclonal antibodies from humans immunised with the vaccine StaphVAX® (described in 8.2.1), and targets CP5 and CP8. Bacteraemic patients were passively immunised with two i.v. injections, next to standard antibiotic treatment. The median time to resolution of *S. aureus* bacteraemia was not significantly different between the AltaStaph® group and the placebo group. The time to hospital discharge was reduced in the AltaStaph® group. However, due to the small sample size, this study was not powered to show efficacy, and therefore, this passive immunisation strategy warrants further investigation [80].

8.2.5. Patients with deep-seated infection

Treatment failure of deep-seated MRSA infections often occurs. Higher vancomycin concentrations are thought to be necessary. However, this results in a higher risk of nephrotoxicity [81]. Therefore, alternatives for vancomycin treatment are urgently needed.

Aurograb® is a single-chain monoclonal antibody variable fragment binding to an ABC transporter. Patients with deep-seated MRSA infection were passively immunised twice daily via the i.v. route, next to vancomycin. No additional efficacy was observed in patients treated with both antibiotics and Aurograb®, and therefore Aurograb® was not further developed [69,82].

8.2.6. Very low birth weight infants

S. aureus is one of the most common organisms causing late onset sepsis, the leading cause of death in infants with very low birth weight [83]. More adequate treatment of this infection is needed to lower mortality.

Veronate® (INH-A21) is an intravenous immunoglobulin from donors with high levels of antibodies directed against ClfA. Premature infants were passively immunised with four i.v. infusions of this IGIV. Bloom *et al* [84] showed that this IGIV had potential to reduce *S. aureus* sepsis and mortality in this group, but statistical significance was not reached. In a study of DeJonge *et al* [85], Veronate® failed to reduce the incidence of late onset sepsis in premature infants.

AltaStaph® (described in 8.2.4), polyclonal antibodies targeting CP5 and CP8, was applied in very low birth weight neonates. Passive immunisation with two i.v. infusions of AltaStaph® induced high levels of CP5 and CP8 specific antibodies, but rates of *S. aureus* bacteraemia were similar in the treatment and the placebo group. However, due to the small sample size, this study was not sufficiently powered to detect small differences in outcome, and therefore a study including more patients is needed [86].

Pagibaximab® is a humanised monoclonal antibody targeting LTA. Patients were immunised twice via the i.v. route. This antibody was shown to be safe and tolerable in very low birth weight infants [87]. Its efficacy has not been evaluated until now.

9. Concluding remarks

Antibiotic treatment of *S. aureus* infections is not always successful. Due to the emergence of antibiotic resistance, alternatives for antibiotic treatment are urgently needed. Active and passive immunisation could be alternative, non-antibiotic related, antimicrobial intervention strategies. Various studies in animal models and humans have been conducted in this field. In animal models, a study design focused on a clinically-relevant infection, a proper bacterial target and well-considered dosing will enhance the translational value of the results obtained.

A range of experimental models of *S. aureus* infections are described to study the efficacy of active or passive immunisation as antimicrobial strategy. The animal models are well selected, as they represent the most common *S. aureus* infections in the clinical setting. A broad panel of *S. aureus* targets has been studied in these experimental models. Active and passive immunisation targeting these *S. aureus* antigens have mostly shown to be successful in prevention, reduction or cure of infection.

In contrast to the numerous studies in experimental animals, only a limited number of clinical studies in *S. aureus* infected patients or patients at high risk for *S. aureus* infection has been performed. In most of these clinical studies, *S. aureus* bacteraemia/sepsis is a common infectious complication, and is therefore used as outcome parameter for efficacy in immunisation studies.

Clinical Phase II/III studies have been performed focused on the bacterial targets CP5, CP8, IsdB, ClfA, ABC transporter, or whole cell *S. aureus*. Successful results were obtained in patients receiving haemodialysis, who are at high risk for *S. aureus* infection. Active immunisation with CP5 and CP8 (StaphVAX®) resulted in a reduction of *S. aureus* bacteraemia. Similar observations were obtained in the experimental *S. aureus* sepsis model in mice, showing that active immunisation with CP5 improved the survival of mice. This indicates the translational value of the results obtained in this experimental sepsis model in mice to the clinical setting.

Other studies in patients with *S. aureus* bacteraemia and in very low birth weight infants who are at high risk for *S. aureus* sepsis, investigating the efficacy of passive immunisation with antibodies targeting CP5 and CP8 (AltaStaph®), were less successful in providing protection against *S. aureus* bacteraemia. Lack of power because of the low sample size might contribute to the lack of success of immunisation. In contrast, in the experimental *S. aureus* bacteraemia model in mice, passive immunisation with antibodies targeting CP5 and CP8 has been shown to protect mice against bacteraemia. Based on these encouraging results in this animal model, more extended studies on the efficacy of AltaStaph® in patients with *S. aureus* bacteraemia are warranted.

Many other *S. aureus* targets have been selected for immunisation studies in animal infection models. In the experimental bacteraemia and sepsis models in mice, successful results were obtained with active or passive immunisation focusing on IsdA, IsdB, SdrD, SdrE, Cna, FnbpA, FnbpB, SEA, SEC, hypothetical protein 2160, ClfA, PNAG, AIP-4, IsaA, α-toxin, and/or ABC transporter, or whole cell *S. aureus*. From these bacterial targets, only IsdB, ClfA, ABC transporter or whole cell *S. aureus* were investigated in clinical Phase II/III immunisation studies. Efficacy was never observed in these studies.

Until now, in most clinical studies immunisation as antimicrobial strategy was investigated in relation to *S. aureus* bacteraemia/sepsis. Based on the successful immunisation studies in experimental animal models of other *S. aureus* infection, such as skin/wound infection, keratitis, mastitis, catheter-related infection, endocarditis, renal abscess, pneumonia and arthritis, studies in patients with *S. aureus* infections other than bacteraemia/sepsis investigating the efficacy of immunisation as antimicrobial strategy seem warranted.

Abbreviations AAHSA, amorphous aluminium hydroxyphosphate sulphate adjuvant; AIP-4, autoinducing peptide 4; Atl; major autolysin; Aur, aureolysin; CFA, complete Freund's adjuvant; ClfA and ClfB, clumping factor A and B; Cna, collagen adhesin; CP5 and CP8, capsular polysaccharide 5 and 8; Eap, extracellular adhesion protein; Efb, extracellular fibrinogen binding protein; FmtB, factor effecting methicillin resistance; FnbpA and FnbpB, fibronectin-binding protein A and B; Geh, glycerol ester hydrolase; GlpQ, glycerophosphoryl diester phosphodiesterase; HlgB, γ hemolysin B; IFA, incomplete Freund's adjuvant; IgG, immunoglobulin G; IGIV, immunoglobulins for intravenous use; IsaA, immunodominant staphylococcal antigen A; IsdA, IsdB and IsdH, iron-responsive determinant A, B and H; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; Lip, lipase; LTA, lipoteichoic acid; LukF-PV and LukS-PV, leukocidin F and S; LytM, peptidoglycan hydrolase; Nuc, nuclease; MRSA, methicillin-resistant *Staphylococcus aureus*; PNAG, poly-N-acetylglucosamine; PVL, Panton Valentine Leukocidin; RAP, RNAIII activating protein; *S. aureus*, *Staphylococcus aureus*; s.c., subcutaneous; SEA, SEB and SEC, staphylococcal enterotoxin A, B and C; SCIN, staphylococcal complement inhibitor; SdrD and SdrE, serine-aspartate dipeptide repeat protein D and E; SOPF,

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specified opportunistic pathogen free; SPF, specified pathogen free; SSL1 and SSL5, staphylococcal superantigen-like protein 1 and 5; SspA and SspB, staphylococcus serine protease A and B; TSST-1, toxic shock syndrome toxin 1.

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