Title:

Characteristics of *Staphylococcus aureus* infections to consider in designing an effective vaccine.

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Running title:

Designing a *S. aureus* vaccine

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ABSTRACT

Staphylococcus aureus is a very versatile and adaptable microorganism. It can potentially infect virtually any host tissue. Given the appropriate conditions it can become a life-threatening pathogen, or a commensal colonizer of the nose. Extensive antibiotic use for infection control facilitated the rise of antibiotic resistance, stressing the need for alternate forms of control. Vaccine efforts in other pathogens have proved successful, but so far *S. aureus* candidate vaccines have not been as effective. Here we review *S. aureus* factors involved in pathogenesis that could help develop a successful vaccine, like host nasal colonization and immune evasion factors. An effective multicomponent vaccine could incorporate antigenic fragments from several *S. aureus* proteins, preferably involved in colonization, immune evasion and/or toxicity.

KEYWORDS: *S. aureus*, Vaccine, Nasal colonization, Immune evasion.

INTRODUCTION

Staphylococcus aureus is a natural inhabitant of mammalian skin and certain mucous epithelia. It is an opportunistic pathogen and has the ability to infect virtually every tissue in the body of animals and humans, especially those at risk of infection like wounds, or with diminished immunological protection like secretory glandular tissue (28). It is a very versatile and adaptable organism; it can become pathogenic causing bacteremia or establish a commensal relationship in humans without causing overt disease, as is the case in nasal

colonization. However, given the appropriate conditions each and every strain of *S. aureus* can become a life-threatening pathogen (29).

Infection control usually requires antibiotics; however, their extensive use has facilitated the emergence of strains with antibiotic resistance (21). Resistance to methicillin and vancomycin has been observed in recent years in hospital and community acquired *S. aureus* infections (5, 44). Methicillin resistance in *S. aureus* is mediated by the acquisition of an exogenous gene, *mecA*, that encodes a ß-lactam-resistant penicillin-binding protein (PBP), termed PBP 2a (or PBP2') (22). There are two known types of vancomycin resistance, complete (vancomycin-resistant *S. aureus*, VRSA) and intermediate resistance (vancomycin intermediate resistant *S. aureus*, VISA). The VRSA resistance is mediated via the apparent acquisition of the *vanA* gene that allows synthesis of modified peptidoglycan precursors with decreased affinity for vancomycin. In VISA, genetic mutations that result in production of a much thicker cell wall makes it very difficult for vancomycin to enter the cell (10).

In this review we will present a summary of *S. aureus* nasal colonization and immune evasion mechanisms used to overcome host responses, as well as strategies used in vaccine design. Based in this information, we then suggest desirable characteristics that future vaccine candidates may incorporate in their design.

Nasal colonization

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The association between *S. aureus* nasal carriage and staphylococcal disease was first reported by Danbolt in 1931 (45), which numerous studies confirmed afterwards (48, 53, 54). Several studies including historical medical controls have reported great reductions of surgical site infections in patients pre-treated to remove *S. aureus* from their noses -nasal decolonization- (7, 25). Although nasal carriage is one of the most important risk factors for nosocomial and surgical site infections, randomized controlled trials have failed to confirm a significant reduction in infection rates after nasal decolonization (24). Therefore, clearance of nasal *S. aureus* is not a completely effective method for infection control. It is possible that after nasal clearance, *S. aureus* that reside in other parts of the body are the source of infection. *S. aureus* cells can survive for months on many types of surface (26), and propagate from there through the hands of the patient (or relatives or caregivers) to the site of infection, or even back to the nasal niche by nose picking (52).

Longitudinal studies distinguish at least three nasal carriage patterns in healthy individuals: persistent carriage (about 20%), intermittent carriage (30%), and non-carriage (50%) (14, 47, 54). Persistent carriers have higher single-strain *S. aureus* loads (their "persistent strain") and higher risk of developing staphylococcal infections (34), while intermittent carriers may carry different strains over time (14, 47). Furthermore, after inoculation with a mix of *S. aureus* strains, non-carriers quickly eliminate all strains, whereas persistent carriers eliminate all strains except for their "persistent strain" when it was present in the inoculation mix (33). It is important to notice that non-carriers who become infected from exogenous *S. aureus* strains have a four-fold increased mortality rate compared with *S.*

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aureus nasal carriers (53). The host immune response that kept non-carriers noses free from *S. aureus* is not effective enough to prevent other *S. aureus* infections.

Recently, a study of anti-staphylococcal antibodies profile showed that levels of IgG and IgA against 17 different *S. aureus* antigens were equal in intermittent carriers and non-carriers but not in persistent carriers. This suggests there are only 2 types of nasal carriers: persistent and non-persistent carriers (46).

Nasal carriage patterns are most likely determined by host and bacterial factors. No relation has been observed between carriage rate and seasonality, temperature, or relative humidity (30, 31, 54). Genetic studies have shown that a simple Mendelian trait probably does not explain host carrier states (1, 3, 36). However, there are observed differences in bacterial attachment to the nasal epithelia of carriers and non-carriers that suggest host factors (genetic and/or environmental) can determine carrier state (2). Personal environmental factors probably have a larger influence: carrier states are usually shared among household members and most mothers carry the same strain as their children (36), suggesting that close contact helps adaptation of the pathogen to its host. Even the anatomy of the nose may influence carrier state (9, 38).

In summary, the existence of non-carriers suggest that there is an immune host response, and probably some genetic host factors as well, that is effective in preventing *S. aureus* colonization. Even in carriers, there is a balance between host and pathogen that allows only a specific strain to colonize and prevents colonization from other strains. However, this balance is lost when *S. aureus* manages to thwart host defenses and invades the host. It

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is likely that genetic changes in the strain are partly responsible for the newly developed abilities of the strain to overcome the host immune response (20), probably aided by host changes, like wounds or diminished immune defenses, that facilitate infective processes. It may be possible to elicit an immune response through vaccination that allows the host to defend against invading bacteria, one that mimics the immune response of non-carriers. Studies in host immune responses have identified *S. aureus* molecules that react strongly to sera of non-carriers. Identified molecules are usually involved in immune evasion and colonization mechanisms by *S. aureus* (13, 49).

Immune evasion

Usually after host internalization, a microorganism and its products are taken up by macrophages and other antigen-presenting cells and transported to lymph nodes, where B cells are stimulated to differentiate and secrete antibodies that neutralize toxins and promote more efficient phagocytosis of bacterial cells. Antibodies to *S. aureus* antigens can be detected in all humans, and titers usually rise after infection (13, 16, 40). However, these antibodies and immunological memory seem to be inadequate to prevent subsequent infections, which reflect the great capacity of *S. aureus* to compromise immune responses. *S. aureus* has an impressive number of immune evasion factors to overcome host defense mechanisms (11, 17). It is important to notice that many of these factors have multiple, often redundant roles: if one of them is rendered inactive through mutation or antibody targeting, its function can still be carried away by another redundant factor (Figure 1).

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After the physical barrier of the skin is breached and the bacterium starts to grow inside the host, the innate immune response is activated (50). *S. aureus* is particularly adept in evading innate host defense, as evidenced by the abundance of mechanisms that the bacterium uses to evade killing by phagocytes (17).

Complement activation is part of the innate immune response, and *S. aureus* has several bacterial products that interfere with its function by (i) the recruitment or mimicking of complement regulators, (ii) the modulation or inhibition of complement proteins by direct interactions, and (iii) the inactivation by enzymatic degradation (27). Phagocyte function is also altered by *S. aureus*, expressed *S. aureus* molecules can block phagocyte receptor function. Bacteria may hide from recognition by producing protective coats, such as capsular polysaccharide or biofilm. After ingestion by professional or non-professional phagocytic cells, the bacteria use mechanisms to decrease the efficiency of antimicrobial mechanisms and to survive killing mechanisms. Intracellular persistence provides a protective niche from professional phagocytes and extracellular antibiotics, and can promote recrudescent infection (19). *S. aureus* often produce toxins that lyse phagocytes and superantigen toxins that overstimulate the immune system (11, 17). The tight control of expression is also essential for pathogenesis. The expression of toxins, colonization and immune evasion factors is controlled by complex regulatory networks that include the quorum-sensing *agr* system, transcriptional regulators of the *sar* family, the two-component regulatory systems *ArlRS* and *SaeRS*, and the alternative sigma factor SigB

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(35). A more detailed review of *S. aureus* molecules that contribute to immune evasion or alter host immune function is presented elsewhere (11, 17).

Vaccine designs.

Is a *S. aureus* vaccine feasible? This is not a question with an easy answer. Recovery from a *S. aureus* infection does not appear to confer immunity against subsequent infections, which cast doubts in the feasibility of generating a better protective immune response than the one induced after natural infection. However, work in the prevention of bovine mastitis (32) showed a 50-70% protection level when using killed bacteria combined with α- and β-toxin toxoids, indicating that it may be possible to generate an immune response with an improved level of protection. Since it is not appropriate to use whole killed *S. aureus* vaccine preparations in humans, alternatives have to be found.

Several reviews covering vaccine development have been published (12, 39, 42). Concisely, the few vaccine candidates that have advanced to clinical trials have failed to show positive results, even after having shown excellent results in animal models of infection. Lessons learned in other pathogens might not be transferable to *S. aureus*. For example, vaccines based in capsular polysaccharides of other bacterial pathogens have proven successful (15, 18), whereas in *S. aureus* failed to show protection (43). Important efforts are directed towards creating staphylococcal subunit vaccines (39), although one could argue that a vaccine carrying only one bacterial factor as immunogen might not work due to the ample role redundancy of *S. aureus* molecules (Figure 1).

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Another approach consists of letting the human immune response choose the best target for vaccination (8, 13, 49, 51). Sera of carriers vs. non-carriers, or healthy vs. infected human subjects is probed against a library of expressed *S. aureus* proteins, with the idea of identify potential targets already recognized by a "good" human immune response to be used as candidate vaccines. This approach has its strength in that bacterial proteins recognized by human sera with a high antibody titer and opsonic activity against *S. aureus in vitro*, are more likely to perform better as vaccine components than bacterial proteins not recognized. Its weakness resides in that selected targets are only as effective as the antibodies used to select them. The fact that non-carriers that successfully fend off *S. aureus* from colonizing their noses can still become severely ill from a *S. aureus* infection means their antibodies are not completely capable of excluding *S. aureus* under all conditions.

There is also the possibility that an identified *S. aureus* candidate antigen in its fully functional state is poorly antigenic, resulting in few or no induced antibodies in the host. Mice immunized with fibronectin-binding protein (FnBP) were able to resist an *S. aureus* infection challenge (6, 41), but were unable to block binding of FnBP to host fibronectin. It was found that native FnBP is poorly immunogenic, but after binding to fibronectin, the FnBP-fibronectin complex is actually immunogenic (37). Therefore, induced antibodies were actually recognizing the FnBP-fibronectin complex instead of preventing its binding. It was later found that biologically inactive FnBP fragments were actually capable of inducing antibodies that could recognize native FnBP and prevent its binding to fibronectin, therefore being better immunogens than native FnBP (4, 23) (Figure 2).

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Therefore, it is possible that an effective vaccine could incorporate antigenic fragments from several *S. aureus* proteins, a multicomponent vaccine, preferably involved in colonization, immune evasion and/or toxicity (Table 1). These antigenic fragments would be biologically inactive proteins that could still induce protective antibodies. The absence of biologically active antigens can increase safety and the targeting of multiple *S. aureus* proteins at once decreases the chances of *S. aureus* immune evasion.

CONCLUSSION

A successful vaccination protocol could greatly contribute to *S. aureus* infection control, providing a much needed relief in reliance of antibiotic therapy alone. Also, advances in the field will help develop effective passive immunization protocols, which are useful in cases were prophylactic vaccination is not possible. Much work is still needed, but the field has greatly progressed lately, with a growing number of research groups working in *S. aureus* vaccine development, as observed after a Pubmed search in *S. aureus* vaccines (http://www.ncbi.nlm.nih.gov/sites/entrez/ access date: July 2009). It is likely that *S. aureus* infection control can be achieved soon.

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Table 1. Possible targets for vaccine development

aaa	Autolysin / adhesin from *S. aureus*
atl	*S. aureus* autolysin
aur	Zinc metalloproteinase aureolysin, Aur
bbp	Bone sialo-protein binding protein
cap5 / cap8	Capsular polysaccharide
chp	Chemotaxis inhibitory protein of *S. aureus*
clfA, ClfB	Clumping factor A and B
can	Collagen binding protein
coa	Staphyloccocal coagulase
crtM, crtN	Carotenoid pigment, staphyloxanthin
dltc	DltC, from Dlt operon, DltABCD
eap	Extracellular adherence protein
ebh	extracellular matrix (ECM) binding protein homologue
ebps	Elastin binding protein
ecb	Extracellular complementbinding protein
efb	Extracellular fibrinogenbinding protein
emp	Extracellular matrix protein-binding protein
fbpa	Fibrinogen binding protein
fnbA, fnbB	Fibronectin-binding proteins A and B
hla, hly	Alpha-hemolysin (α-hemolysin)
hld	Delta-hemolysin
hlgA, hlgB, hlgC	Gamma-hemolysin subunits A, B, and C
icaD, icaB, icaC	Polysaccharide intercellular adhesin, PIA
isdA, isdB	Iron-regulated surface determinants of *S aureus*,
lukS-PV, lukF-PV	Leukocidin S-PV and F-PV, Panton Valentine leukocidin
lukD, lukE	Leukocidin D and E
mprF	Multiple peptide resistance factor
pls	plasmin sensitive protein PLS
psm	Phenol-soluble modulinlike peptides
rap	RANIII activating protein
sak	Staphylokinase
sasg	*S. aureus* surface protein G
sbi	IgG-binding protein
scn	Staphylococcal inhibitor of complement
sea, seb, secn, sed, see, seg, seh, sei, sej, sek, sel, sep	Staphylococcal enterotoxins
spa	Protein A
ssl5	Staphylococcal superantigen-like 5, SSL5
ssl7	Staphylococcal superantigen-like 7, SSL7
tst	Toxic shock syndrome toxin-1, TSST1
vnbp	Vibronectin binding protein
vwbp	Von Willebrand factor binding protein
eno	α-enolase, Laminin binding protein

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Figures:

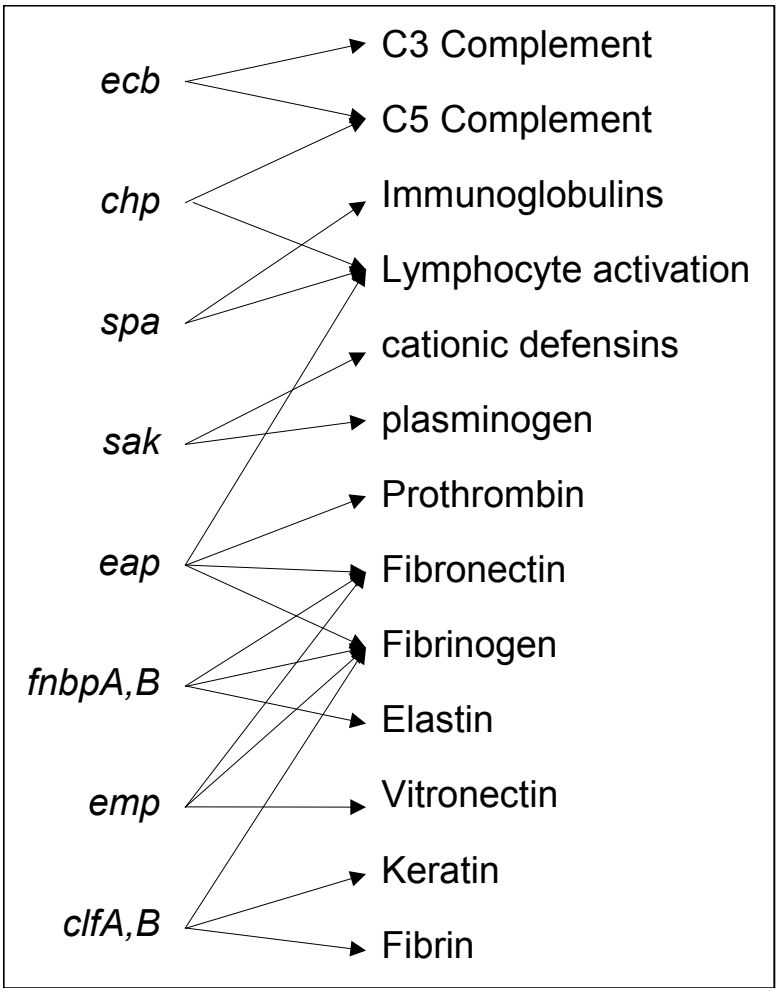

Figure 1. Redundancy in *S. aureus*. Expressed products of *S. aureus* genes involved in pathogenicity, colonization and/or immune evasion usually interact with several targets. Additionally, host factors are usually targeted by different bacterial factors. Redundancy assures infection processes can continue even when some bacterial factors are neutralized by host responses or vaccination

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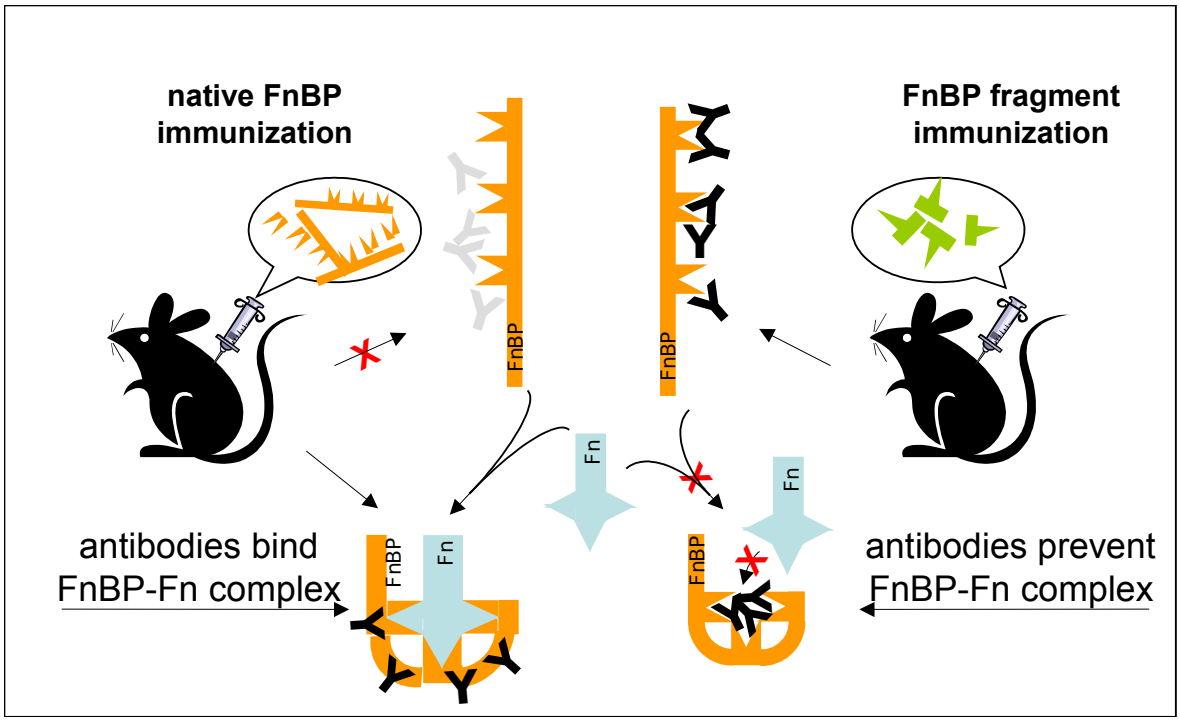

Figure 2. Immunization with FnBP. Immunization with native FnBP induces antibodies that fail to prevent binding of FnBP to Fn, by failing to recognize native FnBP; but recognizes the FnBP-Fn complex. Immunization with biologically inactive FnBP fragments induces antibodies effective in preventing binding of FnBP to Fn, by recognizing native FnBP and failing to bind the FnBP-Fn complex.

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