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Developing a Universal *Staphylococcus aureus* Vaccine: Why Aren't We There Yet?

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Abstract

This review discusses several aspects of vaccine development efforts for *Staphylococcus aureus*. While several vaccines have shown efficacy in pre-clinical models, a potent and successful S aureus vaccine still eludes us. This review captures the historical literature on *S. aureus* vaccine development and discusses the results of major clinical trials in the field. Here we discuss important new insights into immunological mechanisms of protection from extracellular bacterial infections and shed light on important pre-clinical and clinical findings that establish a correlation between *S. aureus* infections and lack of cell mediated immunity. We attempt to provide an immunological explanation for the reasons behind the failure of prior *S. aureus* vaccines and provide insight into the rational design of a universal vaccine for *S. aureus* infections.

Introduction

Staphylococcus aureus is a Gram positive bacterium found as a commensal in humans. Nosocomial *S. aureus* infections are of major concern because of their serious complications, like bacteremia (blood infection), endocarditis, osteomyelitis (bone infection), skin and soft tissue infections *etc*. The overall rate of mortality from *Staphylococcal* bacteremia ranges from 11 to 43 percent [1], primarily due to the emergence of antibiotic resistant strains. *S. aureus* infections are now the most common cause for children being hospitalized for surgical drainage of pus, the most common cause of bacteremia in people over 65 years old, and the most serious cause of prosthetic device and intravascular line infection [2]. Risk factors for infection include disruption of mucosal or cutaneous surfaces, introduction of foreign or medical device, surgery, hemodialysis or host immune suppression. A growing body of data suggests that immunity to *S. aureus* infection does not persist and recovery from infection does not necessarily confer resistance [3]. Thus a vaccine approach is urgently required to address these problems. However unlike several other successful bacterial vaccines, a universal vaccine to prevent *S. aureus* still eludes us. Historically the development of vaccines against several bacterial pathogens like *Haemophilus influenzae* type b, *Streptococcus pneumoniae, Neiserria meningidtis*, and toxigenic bacteria causing diphtheria, pertussis, and tetanus has focused around developing protective neutralizing Ab against the bacterial surface antigens that promote bacterial attachment, invasion or evasion of host immunity and /or toxins that function as virulence factors An evident question that hence comes to mind is why we have vaccines for some but not all bacterial pathogens? Perhaps the answer lies in admitting that until now the contribution of immunology to the development of antibacterial vaccines has been minimal. We have exhausted the list of pathogens that were amenable to vaccine development based on straight forward Ab responses, or trial and error; a comprehensive understanding of the immune response is now needed for rational design of vaccines that are directed at more complex pathogens like *S. aureus*.

Host Immune Response to *S. aureus:* What is Protective?

An important dilemma in the field of *S. aureus* immunology is a lack of understanding of what constitutes protective immunity to these bacteria. Although extensive investigation of the immune responses generated upon vaccination of mice has been conducted, these have not been good predictors of outcome in the human population. Several clinical trials with vaccine (active or passive immunization) candidates aimed at boosting opsonizing Ab titers to the bacterial surface polysaccharides, proteins, or toxins have been unsuccessful or inconclusive [4-8].

Most vaccine constructs elicit humoral responses; however, the role of opsonic Ab in human staphylococcal infection is uncertain, since the titer of anti-staphylococcal Ab does not necessarily correlate with protection [9,2,10]. The failure of several clinical trials based on passive immunization approach [7,8,11,12], once again questions the validity of using only opsonic and/or neutralizing antibody for protection. A common observation from multiple trials has been a lack of understanding of measures of protective immunity, a lack of a well defined predictive animal model and a lack of efficacy in humans, especially in terms of preventing nasal colonization and infection. These outcomes urge for a better rational design of a vaccine via detailed understanding of the entire spectrum of immune responses generated to these bacteria.

It is well known that although healthy persons naturally have high Ab titers to *S. aureus* and also patients with natural defects in humoral immunity are not particularly prone to *S. aureus* infections. For example, patients with hereditary agammaglobulinemia (X linked agammaglobulinemia) rarely have clinically important *S. aureus* infections, although *H. influenza, S. pneumoniae* and *Pseudomonas* species are clinically important in these patients [13-15] and often lead to death. Correspondingly, opsonizing Ab based vaccines have worked well against *S. pneumonia* [16] and *H. influenza* [17]. Thus the lack of Ab against *S. aureus* in patients with agammaglobulinemia must be compensated for by other immune mechanisms. This apparent lack of Ab contribution to *S. aureus* disease prevention is somewhat surprising since theoretically, both Ab and complement facilitate opsonophagocytic killing (OPK) activity by human neutrophils, which can enhance the bactericidal effect of neutrophils. However, while the

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role of neutrophils in combating a *S. aureus* infection is substantiated by numerous studies on human patients as well as mice, the role of opsonic Ab remains uncertain. For example, as opposed to the outcome of *S. aureus* infection in patients with hereditary agammaglobulinemia, patients with inherited conditions that lead to neutrophil dysfunction, such as chronic granulomatous disease, SCN (severe congenital neutropenia), or Chediak- Higashi disease suffer from an increased incidence of *S. aureus* infections [18-21].

Two complement components, namely C3 and C5, also play important roles in clearance of *S. aureus*. Individuals with C3 or C5 deficiencies suffer from repeated pyogenic infections including pneumonia, sepsis, and sinusitis. *S. aureus* is an important cause of infections in these individuals [22,23]. Complement components C3 and C5 can opsonize bacteria, and cleavage products C3a and C5a are anaphylatoxins which attract neutrophils to sites of bacterial infection. Patients in which liver function is compromised due to cirrhosis, have abnormally low levels of

C3 and experience repeated infections due to *S. aureus*. Interestingly the concentration of C3 appears to be more predictive of morbidity in these patients than the opsonizing activity of this serum factor [24]. This could be due to production of C3a and/or other C3 degradation products.

The role of opsonic Ab in mediating neutrophil bactericidal activity also becomes doubtful when considering a recent report that human Abs to distinct *S. aureus* surface antigens exhibited mutual interference, which neutralized opsonic killing and protection in mice [25]. These data could be highly relevant in the rational design of a *S. aureus* vaccine as interference or neutralization of opsonic activity by pre-existing antibodies could potentially be the reason for lack of effective boosting of immunity via a vaccine to otherwise immunogenic antigens. Thus the current paradigm that human immunity to extracellular Gram positive bacterial pathogens is primarily mediated by opsonophagocytic killing (OPK) via Ab specific for surface antigens, needs additional scientific clarification. In fact, several sources of data indicate that while bactericidal neutrophil activity is essential in resolving a *S. aureus* infection [26], the conventional Ab mediated opsonophagocytic uptake mechanism may not be primarily responsible for enhancing the bactericidal activity of neutrophils [27]. It has been reported that neutrophils are killed under *in vitro* conditions, by phagocytosed *S. aureus* [28]. Additionally, Ab may enhance bactericidal activity by methods other than through opsonization and binding to phagocyte Fc receptors [29-31]. Growing evidence points towards an alternative immune mechanism which leads to stimulation of neutrophils in combating extracellular bacterial pathogens. Our recent

Knowledge of the role of IL17 producing T helper cells, namely Th17 cells, for protection against *S. aureus* infections appears to be the missing link in this conundrum [32].

Emerging Elucidation of the Role of Cell Mediated Immunity in *S. aureus* Infections

Some of the earliest claims for a role of T-cell mediated protective responses (CMI) in *S. aureus* pathogenesis were authored by who used a mouse model of *S. aureus* arthritis to show that neither IgG nor B cells (humoral) were "required" for protection from disease and/or bacterial clearance [33-35]. These studies were noteworthy as they claimed a primary role for the cellular arm of the immune system in combating *S. aureus* infections. Using IL4 deficient mice in the same model they also showed that mice lacking this prototype Th2 cytokine had decreased septic arthritis and arthritis mediated mortality [35]. These results are highly relevant considering the suggestion that skewing the immune response towards the Th2 type can exacerbate disease (and mortality) in this model by preventing bacterial clearance. Thus type 1 T helper cells (Th1) which initiate primarily a cellular immune response by recruiting macrophages and secreting IFN-γ and IL-12 were implicated as being important. Several recent studies have now started to describe the role of CMI in *S. aureus* infection, albeit with different and novel players. A new subset of Th cells, aka Th17 cells, has now been shown to be of growing importance in a *S. aureus* generated immune response. The cytokine IL17 produced by this Th subset is implicated to have a distinct role in the neutrophilic activity involving *S. aureus* [36,37]. IL17 is a recently discovered family of cytokines (IL17A-F) produced primarily by CD4+ T (helper) cells. Other innate immune cells like γδ T cells, natural killer (NK), NKT cells can also produce this cytokine to some extent. IL17 A and F are the most closely related members of this class and also the best studied. IL-17 has profound effects on neutrophils, including induction of granulopoiesis and chemotaxis through stimulation of structural cells such as endothelial and epithelial cells, also the production of granulocyte stimulating factor (G-CSF), macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) [38]. IL-17 has been shown to directly mediate neutrophil apoptosis and phagocytosis of apoptotic neutrophils by macrophages [39]. Taken together these studies indicate that IL-17 has the capacity to control not only the recruitment, but also the total turnover and function of neutrophils. Thus considering the known importance of the bactericidal role of neutrophils against *S. aureus*, it is but obvious to examine the relationship among IL17 (and Th17 cells), neutrophils and *S. aureus*.

Recent work in murine models has revealed a role for IL17A in combating *S. aureus* infections. Using genetically engineered IL17A KO mice, showed that IL17A is essential for clearance of murine mucocutaneous infections caused by *S. aureus* [37]. Similarly Lin et al extended this knowledge to blood stream infections of *S. aureus* and *C. albicans* through investigation of an anti-fungal protein that cross reacts with *S. aureus* [40]. Protection against *S. aureus* challenge following rAls3p-N (a cross protective fungal antigen from *Candida albicans*) immunization was found to be mediated by T cells, specifically Th17 and Th1. Of note, this protection was lost in mice that lacked neutrophils producing reactive oxygen species, i.e., gp91phox$^{-/-}$ mice. We have also recently demonstrated that Th17/IL-17 immunity is determinative in IsdB immunized mice challenged with *S. aureus* [32]. We reported that IsdB immune T cells were critical for protection of SCID mice challenged with a lethal dose of *S. aureus* via the tail vein, while neither active immunization nor passive transfer of monoclonal antibodies (mAb) against IsdB provided protection to the SCID mice. This result implicated T- cells as being important for protection. Adoptive transfer of IsdB immune CD3/4+ cells protected the SCID mice whereas neither CD8+ T cells, nor B cells, nor plasma cells were protective. Immunization with IsdB was shown to be critical for activating the transferred T cells, as T cells from BSA immunized mice were not protective. To dissect the immune response further, it was shown using intracellular staining that IsdB immune CD4+ cells stimulated with IsdB produced IL17, and that IL17, but not IL22 nor IFNγ was necessary for IsdB-generated protective immunity. Finally, the Th17 immunity was specific for IsdB because challenging mice replenished with IsdB immune T cells with an *isdB/harA* (HarA is an IsdB homolog) deleted strain did not result in protection. Thus, IsdB vaccine mediated murine protective immunity to *S. aureus* infections is specific and dependent upon Th17/IL17.

Interestingly, the observations made with these animal experiments reflect clinical information from the human patient population. For example, patients with Job's syndrome who have a genetic defect in STAT3 signaling and thus the lack of Th 17 cell development or people with IL17 defect are prone to *S. aureus* skin infections [19]. Similarly patients with defects in cell mediated immunity (eg, HIV/AIDS, steroid therapy etc) present an increased incidence of *S. aureus* skin and mucocutaneous infections [41]. Thus these observations are consistent with mice data implicating the crucial role performed by CD4+ Th17 cells in protection from naturally occurring skin and soft tissue infections [37,42]. Thus far, the Th17 response to one human *S. aureus* vaccine has been reported. The recombinant Als3p-N based vaccine with cross protection to *Candida albicans* and *S. aureus,* being developed by Novadigm has been reported to stimulate Th17 response after vaccination [40]. Data from a phase I safety and immunogenicity trial indicated that patients responded to a single dose of the antigen, with increases in Ab titers, Th1 and Th17 stimulation [43,44]. Human Th17/IL17 responses to other *S. aureus* vaccines in recent development are not currently known. Murine data indicates that vaccines designed to prevent *S. aureus* infections may need to contain Th17 stimulating antigens and/or be administered with a T cell stimulating adjuvant. While Ab mediated protection is clearly dispensable in a murine model of vaccine efficacy, the role of Ab in a human vaccine is still unclear. Whether Ab alone are ineffective or just insufficient for providing protective immunity in a human vaccine is still an open question.

Clinical Trial Outcomes for *S. aureus* Vaccines

Most active or passive vaccination methods tested in clinical trials till date have aimed at eliciting or boosting specific humoral responses, and these have been not been successful in the clinic [45-47]. An important point to note here is that antigen targets were selected based primarily on ability to enhance levels of opsonophagocytic and/or neutralizing Ab. The results from the clinical trials showed that the titer of anti-staphylococcal (surface antigen or toxin) Ab did not correlate with protection, as measured by reduction in bacteremia and hence the role of opsonic and/or neutralizing Ab in human staphylococcal infection remains uncertain. Given the fact that humans have pre-existing Ab titers against most *S. aureus* virulence and pathogenicity factors, it is not apparent that merely increasing these titers through vaccination will lead to protection. Therefore one can ask whether vaccines stimulating solely the humoral immune system are sufficient for preventing disease. *S. aureus* surface capsular polysaccharide (CP) was tested as a potential vaccine candidate based on the sero-epidemiologic evidence that shows the presence of anti–CP Ab in human sera [48]. After protection from infection was demonstrated in several animal models, with anti-CP Ab [45,49,50], the most prevalent serotypes CP5 and CP8 were tested in a human clinical trial [51,52]. It was found that CP is weakly immunogenic and in order to achieve efficacy, conjugation to a carrier molecule was required. In fact *S. aureus* CP5 conjugated to *Pseudomonas aeruginosa* exotoxin A protected mice against challenge infection with homologous serotype [45,53]. Antibodies generated to bivalent CP5 and CP8 conjugated to exotoxin A vaccine were also protective in a rat catheter infection model [49]. When tested in a Phase I/II, safety and immunogenicity trial, the bivalent CP5/8 Ps-exotoxin A conjugate vaccine (StaphVAX, NABI) did evoke type specific, opsonophagocytic Ab in healthy adults [51]. However when tested for efficacy in patients with end stage renal disease receiving hemodialysis, StaphVAX did not evoke consistently high levels of Ab and serum concentration fell over time post vaccination [6]. The trial showed some efficacy from week 3 to week 40, but mortality actually increased during the later stage of the trial so that there was not a net protective effect. The resulting data from this trial were confusing at best, as there were claims of a reduction of bacteremia at certain intervals within the study, in the face of ever declining serum Ab. In a second larger study the investigators identified a booster effect when patients were re-vaccinated after 2-3 years, however there was no net protection from bacteremia. Thus a direct correlation between Ab response and protection was not found in this study either. A multi-component vaccine containing protein conjugates of CP5, CP8, teichoic acid, alpha-toxin and Panton-Valentine leukocidin (Penta Staph) is currently being evaluated [54]. An approach using passive immunization with polyclonal immunoglobulin (Ig) against CP5/CP8 exotoxin A conjugate (AltaStaph®, NABI) was also tested in the very low birth weight infant population. This vaccine was well tolerated but it failed to statistically reduce *S. aureus* bacteremia [55]. In a later study the administration of Alta Staph® as adjunctive to antimicrobial therapy failed to improve survival in patients with *S. aureus* bacteremia [56], thus ruling out the efficacy of capsule induced Abs in protection against *S. aureus* bacteremia.

S. aureus surface adhesins have also been evaluated as vaccine targets. The surface adhesin lipoteichoic acid (LTA) is particularly interesting as it binds to target cells both non-specifically, e.g., to membrane phospholipids, and specifically, e.g., to CD14 and to Toll-like receptors (TLRs). LTA can interact with circulating Ab and activate the complement cascade to induce an innate bactericidal effect. Soluble LTA also triggers neutrophils and macrophages to release reactive oxygen and nitrogen species, acid hydrolases, highly cationic proteinases, bactericidal cationic peptides, growth factors, and cytotoxic cytokines, which may act in synergy to amplify cell damage [57]. Thus, LTA shares many of its pathogenic properties with bacterial endotoxins like lipopolysaccharide (LPS). In animal studies, soluble LTA has induced arthritis, nephritis, uveitis, encephalomyelitis, meningeal inflammation, and periodontal lesions. LTA can also trigger cascades resulting in septic shock and multi-organ failure [58]. Binding of LTA to targets can be inhibited by LTA specific Ab, by phospholipids, and by specific Ab to CD14 and Toll receptors [59]. I*n vitro* release can be inhibited by non-bacteriolytic antibiotics and by polysulphates such as heparin, which probably interfere with the activation of autolysis. LTA is highly conserved among Gram positive bacteria. Based on this evidence, LTA can be considered a virulence factor that plays an important role in infections and in post infectious sequelae caused by Gram-positive bacteria, thus spurring interest as a vaccine candidate [60]. A mouse chimeric mAb against LTA (Pagibaximab®) was evaluated for clinical use in very low birth weight infants [55,61]. The trial was discontinued, due to lack of efficacy in this population. The study failed to demonstrate protection against *S. aureus* bacteremia, in the first 35 days of administering the mAb to LTA (www.clinicatrials.gov; STUDY- NCT00646399).

Multiple microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) have been studied [3,62] as vaccine targets. These molecules (including clumping factor A and B (ClfA, ClfB), collagen binding protein (CNA) and fibronectin binding proteins A and B (FnbpA , B) play a key role in bacterial adhesion to extracellular host matrix proteins, and thus initiate a *S. aureus* infection by facilitating colonization and invasion of host tissue by the bacterium. These antigens were partially protective in laboratory animals [63-65] as a result; the antigens have been the target of immunotherapy approaches. A polyclonal preparation of IVIG containing high titers to *S. auerus* ClfA and anti-*S. epidermidis* SdrG (fibrin-binding protein), Veronate® (IHN-A21, Inhibitex), was tested in low birth weight neonates for prevention of late- onset sepsis, and

the IVIG lacked efficacy against this study end point [8]. A humanized mAb recognizing *S. aureus* clumping factor A,

Aurexis® (Tefibazumab, Inhibitex), was evaluated in 60 patients with documented *S. aureus* bacteremia. They received either Tefibazumab or a placebo in addition to antibiotics with the intent of enhancing the efficacy of vancomycin therapy. Once again, there was no significant difference in relapse of bacteremia, complications related to the *S. aureus* bacteremia, or death, between the treated and untreated groups in the clinic [46] despite showing protective efficacy in a murine sepsis and a rabbit endocarditis model of infection [66,67]. Thus while these antigens are conceptually interesting vaccine targets, Ab to these candidates have yet to demonstrate significant protective efficacy in human clinical trials. An important point to note is that many MSCRAMMs bind to components of extracellular matrix that are extremely similar in molecular structure. Thus it is very likely that blocking one MSCRAMM by passive immunization may still allow the bacteria to attach to the host cells, using other similar motifs.

Another *S. aureus* polysaccharide studied as a vaccine candidate is the de-acylated form of intracellular adhesin poly-N-acetyl-glucosamine or dPNAG. dPNAG conjugated to diptheria toxin was shown to evoke opsonophagocytic Ab that protected mice from *S. aureus* challenge infection [68,69]. Mice passively immunized with anti-dPNAG –DT rabbit sera had increased clearance of *S. aureus* from blood as compared with controls immunized with normal sera. Overall, the data showed an enhanced immunogenicity of PNAG upon deacetylation and coupling with a protein moiety. A human mAb to dPNAG (MAb F598) has been developed for clinical use. After a phase I clinical safety evaluation in the healthy adult population was successfully completed, a Phase II randomized, double-blind, placebo controlled trial to assess the pharmacokinetics, pharmacodynamics and safety of MAb F598 in mechanically ventilated patients in the ICU was initiated. While the results were eagerly awaited, the trial had to be terminated due to lack of appropriate patient population recruitment (http://clinicaltrials.gov; STUDY- NCT01389700).

Secreted toxins from *S. aureus* have been evaluated as vaccine candidates, thus far mainly in animal models. Alpha-hemolysin (Hla), Panton–Valentine leukocidin (PVL), toxic shock syndrome toxin (TSST-1) and staphylococcal enterotoxin have all been evaluated as active and passive immunization targets, although with mixed results [70-73]. Much interest was generated in Hla when antibodies against Hla were found to prevent injury to infected human lung epithelial cells during *in vitro* culture [74]. When investigated as a vaccine candidate in an *in vivo* murine sepsis model, active immunization with a single amino acid mutant of Hla (H35L) generated antigen specific IgG and caused significant decline in mortality of mice post intranasal challenge, as compared to controls [74]. The requirement for Hla expression for pathogenesis during pneumonia was demonstrated in a murine model and hence an association between Hla expression in *S. aureus* strains, and virulence was established. However, other studies in rabbits have shown that high circulating Hla Ab levels reduced the lethal effects of the toxin but did not prevent *S. aureus* induced abscess formation or reduced bacterial burden [75]. These data then suggest that Ab blocking of alpha toxin may help prevent severe disease but would be less useful in preventing sub-lethal disease or colonization.

Recently, the antigen IsdB has been clinically characterized as a potential vaccine candidate. Lessons learned from development of this vaccine antigen could give insight in the rational design of a future *S. aureus* vaccine. IsdB is a cell wall anchored protein presumably involved in iron scavenging by the bacteria [76]. It is one of the proteins in the iron regulated surface determinants (Isd) locus that are considered important for *S. aureus* survival in the human host [77]. *In vitro* assays demonstrated that IsdB specific mAb could enhance the OPK activity of HL60 cells, a predominantly neutrophilic promyelocyte cell line. Additionally vaccination of mice with IsdB reduced kidney abscess formation [78,79] and formation of early biofilm on indwelling rat catheters. IsdB specific murine and human mAb mediated OPK activity optimally at 100 - 200 μg/mL [80]. Passive immunization of mice with human IsdB specific mAb, CS-D7, enhanced survival at 17-20 mpk in a murine sepsis model [81], and reduced catheter colonization at 12-13 mpk in a rat indwelling catheter model [82] against a very high challenge dose (~2×10^9 CFU) in each case. Based upon extensive supportive preclinical data, IsdB was investigated for the prevention of clinical *S. aureus* infections. Antibody titers to IsdB were shown to correlate with protection in several preclinical models including sepsis and indwelling catheter models and were therefore monitored as a biomarker of vaccine efficacy. Importantly, in phase I testing, vaccination with a single dose of immunogen induced an anamnestic Ab response to the vaccine, and by day 14 post immunization 86-87% of patients vaccinated (with either 30 or 90 μg V710) had Ab titers of twice the pre-vaccination level (geo mean of either 116 μg/mL or 131 μg/mL IgG respectively) [83]. Further phase I testing led to the selection of a 60-μg dose of lyophilized, non-adjuvanted antigen for efficacy evaluation. A pivotal sequential-design trial was conducted to assess safety and efficacy against bacteremia and deep sternal wound infections in patients undergoing cardiothoracic surgery. Patients were vaccinated 14-60 days prior to surgery, and monitored for safety and *S. aureus* infections for 90 days post-surgery. Patient safety and vaccine efficacy were monitored by a Data Monitoring Committee (DMC) at pre-specified interim time points in the trial. At the second interim analysis, the DMC recommended to terminate the trial due to a low probability of achieving vaccine efficacy as well as a safety concern regarding overall increased mortality and multi-organ dysfunction that occurred with greater frequency in vaccine recipients, compared with placebo recipients [47]. Evaluation of immune response to V710, indicated patients administered V710 had a robust Ab response to IsdB, equivalent to that observed in multiple phase I trials. The T cell response to the vaccine was not determined.

In considering the repeated efficacy failures of the multiple *S. aureus* vaccine trials, we can perhaps find a common denominator to help explain these results. This pertains to the Th17/IL17 mediated cellular immune response to *S. aureus* infections. We have generated data to indicate that IL17A plays a critical role in protection against *S. aureus* in a disseminated infection model [32]. Other authors have shown IL17A mediated resolution of *S. aureus* cutaneous infections [37], as well as implicated involvement in the clearance of *S. aureus* from lungs upon co-infection with influenza A. [84]. Importantly, the link between defects in STAT3, lack of IL17, and recurrent *S. aureus* infections, as observed in Job's disease patients, has lead to an important understanding of IL17 involvement in *S. aureus* infection and pathology [19]. Perhaps inclusion of appropriate target antigens, and adjuvants, which stimulate T cells, particularly Th17 cells, must be considered in the rational design of the next *S. aureus* vaccine.

Conclusion

In summary, multiple vaccine trial outcomes imply that designing a universal *S. aureus* vaccine will be a challenging endeavor, for several reasons. Firstly, we do not understand what constitutes protective immunity in the human host, although it is clear from the repeated failures of past clinical trials that Ab may not be the best correlate of

protection. In fact the results of the recently failed clinical trials beg us to dig deeper. Focusing upon opsonophagocytic Ab against *S. aureus* has not resulted in an effective vaccine (e.g. StaphVax). Because humans live in frequent contact with *S. aureus*, and have a high rate of nasal colonization, all normal people have developed natural *S. aureus* Ab which have potent opsonizing activity [10]. Our focus on Ab mediated immunity has stemmed from vaccine success stories of the past, like tetanus or diptheria where serum Ab neutralize dangerous toxins, or pneumococci and hemophilus where Ab activate phagocytes and complement (OPK) to kill the bacteria. Our limited knowledge of the definitive mechanism of immune protection from *S. aureus* has resulted in the development of vaccine candidates that cannot boost significant additional protection on top of what naturally exists in most healthy people. Perhaps we need to change our conventional thinking of designing bacterial vaccines based on pre-existing Ab in the serum, which prevent disease but not infection. A rational vaccine design should take into consideration the fact that both humoral immune response mediated by Ab and cellular immune response mediated by T cells are controlled by T helper cells. Thus going forward this subset of T cells should be in the fore front of rational vaccinology. Additionally, animal models have done little in predicting protective immunity, thus indicating that a suitable animal model for studying *S. aureus* infections in humans still eludes us. Secondly, a universal vaccine may be tough to achieve given that *S. aureus* infected patients present a wide array of diseases that range from muco-cutaneous to soft tissue to systemic infections. As we understand now, the native immunity of humans to the bacterium is modulated according to the site of infection. Thus designing a universal vaccine presents the additional challenge of modulating the host immune response as per the site of infection. Given our limited understanding of protective immunity to this bacterium, this may be a difficult challenge. Lastly the commensal nature of the pathogen renders it with several pathogenicity factors that neutralize host immune response and dampen the effects of vaccine generated immunity. Thus in light of these facts we need to base our approach to a rational design of a universal *Staphylococcus aureus* vaccine from the very beginning, on a greater understanding the immunological correlate of protection.

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