Human Vaccines 6:10, 857-859; October 2010; © 2010 Landes Bioscience

A new view on development of a *Staphylococcus aureus* vaccine

Insights from mice and men

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Key words: S. aureus, vaccine, immunology, cell mediated, Th1, Th17, humoral

Submitted: 05/19/10

Accepted: 05/19/10

Previously published online: www.landesbioscience.com/journals/vaccines/article/12469

DOI: 10.4161/hv.6.10.12469

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When rationally designing a vaccine against a specific pathogen, several categories of microbiologic and immunologic data can offer insight. The identification of the protective antigen is of paramount importance. Another is the nature of the immune response to natural infection, which presumably is protective against recurrent infection caused by the same pathogen. A third category concerns the insight that is sometimes gained from understanding the nature of host defense immunologic defects that predispose to, or increase the severity of, the pathogen in question.

Based on these principles, the design of vaccines targeting toxin-mediated diseases is relatively straightforward: immunize with a detoxified toxin analog to generate neutralizing antibodies (e.g., diphtheria, pertussis, tetanus toxoid). Similarly, for viral diseases, successful vaccines based on whole virus (e.g., Hepatitis A Virus), viral antigens (e.g., Hepatitis B Virus) or viral-like particles (e.g., Human Papilloma Virus) have been designed to generate antibodies that block viral interaction with host cells, and induce cytotoxic T lymphocytes to kill virally infected host cells.[1] In these examples, the vaccines are designed to induce an immune response which mimics protective immunity against natural disease caused by the target toxin or pathogen, and also stimulates mechanisms of host defense which, when absent, predispose to the target disease.

Similarly, the underpinning of vaccination against encapsulated bacterial organisms is based in part on the well-described hypersusceptibility to these organisms of patients with congenital or acquired B cell/antibody deficiencies, as well as the same hypersusceptibility of animal models in which B cell/antibody function is disrupted.[2] These clinical and experimental immunologic observations are further bolstered by data establishing that antibody concentrations correlate with protective immunity after natural infection caused by such encapsulated organisms. Hence, vaccines against encapsulated bacterial pathogens are designed to stimulate antibodies that neutralize the anti-phagocytic capacity of the polysaccharide capsule of *Streptococcus pneumoniae*, *Hemophilus influenzae* type B and *Neisseria meningitidis*, enabling the host immune system to clear the organism.[1]

After a half century of successful vaccine development for toxin, viral and encapsulated bacterial diseases, it is perhaps not surprising that initial efforts to develop a staphylococcal vaccine was based on the same immunologic mechanism: a humorally focused vaccine. The leading effort in this regard was StaphVAX, a bivalent vaccine comprised of *S. aureus* capsular polysaccharide types 5 and 8 bound to pseudomonal exotoxoid A as a carrier. In phase II clinical trials, the vaccine resulted in high antibody titers that lasted for approximately 6 months in patients undergoing chronic hemodialysis.[3-6] Furthermore, a booster dose appeared to maintain antibody levels for more than a year. Unfortunately, in a large, pivotal phase III trial, StaphVAX did not reduce the incidence of invasive *S. aureus* infections in hemodialysis patients.[7] This lack of protective efficacy

occurred despite the presence of impressive anticapsular antibody concentrations in immunized patients. Another factor limiting this approach is that many clinical isolates lack these capsular types or indeed any capsule. For example, the major genetic background causing epidemic community acquired (CA) methicillin resistant *S. aureus* (MRSA) infection, USA300, elaborates no detectable capsular polysaccharide.[8]

Passive vaccine strategies targeting *S. aureus* have also been developed. The Aurexis™ anti-staphylococcal monoclonal antibody targets the Microbial Surface Components Recognizing Adhesive Matrix Molecule (MSCRAMM), clumping factor A. A phase II clinical trial of Aurexis™ as adjunctive therapy in patients with established *S. aureus* bacteremia resulted in an insignificant trend towards improved outcomes for treated patients.[9] However, high-titer anti-clumping factor A polyclonal antibody resulted in no clinical benefit among high-risk premature neonates, and did not reduce the risk of developing invasive staphylococcal infection.[10]

The failure of an active, polysaccharide capsular-based vaccine despite successful induction of opsonophagocytic antibodies, combined with the failure of passive immunization against *S. aureus* in clinical trials, highlights a logical disconnect between these humoral-based strategies deployed against *S. pneumoniae*, *H. influenzae* and *N. meningitidis* versus a similar approach against *S. aureus*. In contrast to toxins, viruses and encapsulated bacteria, to date, no study has defined the nature of protective adaptive immunity that occurs after natural infection by *S. aureus*. Indeed, it is not at all clear that natural infection by *S. aureus* leads to an immune response that protects against re-infection. In the absence of available data on protective immunity after natural infection, the only data available on which to base a rational vaccine program from *S. aureus* are derived from clinical and experimental deficiencies in specific host defense mechanisms which predispose to *S. aureus* infections.

The immunopathogenesis of *S. aureus* infections stands in contrast to typical encapsulated bacterial infections. Patients with B cell or antibody deficiency or asplenic states, are not at higher risk for *S. aureus* infections, nor do they have especially severe *S. aureus* infections when such infections occur.[2] The clinical experience with patients was recapitulated in a recent study of mice deficient in B cells, which were not more susceptible to *S. aureus* bacteremia than wild type mice.[11]

Specific risks that appear to predispose to development of uncomplicated skin infections primarily relate to behavioral and hygiene factors that result in increased exposure to *S. aureus*, particularly in the setting of minor trauma to skin.[12-15] The primary predisposing risk factors for acquisition of more invasive staphylococcal infections, and for increasing severity of such infections, are: (1) defects in anatomical barriers, such as from burns, intravenous catheters, urinary catheters, traumatic or surgical wounds, cardiac valvular abnormalities, bronchiectasis/airway disease, etc., and (2) quantitative or qualitative defects in phagocytic function.[2,16-18]

We have found that IFNγ deficient mice are hypersusceptible to infection caused by *S. aureus* inoculated intravenously.[11,19] Others have found that dual IL-17A/F deficient mice had an increased incidence of developing spontaneous skin infections caused by *S. aureus*.[20] Hence, a new immunologic strategy to develop an anti-*S. aureus* vaccine may be to induce memory T cells which are capable of increasing the rapidity and strength of phagocyte recruitment to sites of infection, facilitating clearance of the organism from tissues.

Indeed we have recently described a novel vaccine strategy against *S. aureus* which is based on immunologic cross reactivity of the candidal recombinant N-terminus of Als3p (rAls3p-N) vaccine against *S. aureus* cell wall preparations.[11,19] The immunology of this vaccine offers new insights into immunologic mechanisms by which vaccines may be effective at protecting against invasive *S. aureus* infections. The rAls3p-N vaccine induced high antibody concentrations, but these antibodies were not protective when used to passively immunize against *S. aureus* intravenous challenge.[11] Concentrations of anti-rAls3p-N antibodies in individual mice did not correlate well with the risk of death from staphylococcal infection.[21] Furthermore, the vaccine was equally effective in B cell deficient mice as wild type mice, but had no efficacy in T cell deficient mice.[11] Adoptive transfer of immune $B220^+$ B cells did not transfer protection, but transfer of $CD4^+$ T cells did transfer protection. The vaccine was ineffective in IFNγ and IL-17A deficient mice, and in $gp91^{phox-/-}$ mice that are unable to produce superoxide. These latter mice are therefore used as an animal model for Chronic Granulomatous Disease.[21] Cross-adoptive transfer experiments confirmed that functional phagocytes were operative in vaccine-mediated protection at the downstream effector stage, not the upstream lymphocyte priming stage. Finally, vaccination increased the recruitment and activation of phagocytes at sites of tissue infection in mice, and cytokines produced by vaccine-primed lymphocytes markedly improved the ability of phagocytes to kill *S. aureus*. Hence, the rAls3p-N vaccine demonstrates that it is feasible to induce a protective immune response in mice against *S. aureus* in the absence of induction of protective antibodies, and by inducing a protective Th1/Th17 response.

Clinical and animal model experience has indicated that hosts deficient in phagocytes, or phagocytic function, are specifically predisposed to *S. aureus* infection. This concept strongly suggests that vaccines can be developed to specifically enhance phagocytic-mediated host defense mechanisms against *S. aureus*. Nevertheless, recent experiences confirm that it is possible to induce and identify protective antibodies even against diseases which are clearly not dependent on antibody-mediated protection. Examples include disseminated candidiasis and invasive aspergillosis.[22-24] Therefore, the available immunopathogenesis data do not preclude development of a humoral based vaccine against *S. aureus*. Rather, they suggest that cell-mediated vaccines merit additional focus, and raise the possibility of combining antigens that stimulate both humoral and cellular responses against the pathogenic organism. Indeed, the latter may be the most likely strategy

to result in a strongly protective vaccine against *S. aureus*.

Acknowledgements

B.S. owns shares in NovaDigm Therapeutics Inc., which is developing vaccine technologies against *S. aureus*.

R.D. was a paid consultant for Glaxo Smith Kline, Merck and Wyeth concerning *S. aureus* vaccine development. He currently consults for Gluconaxyn, which is developing vaccine technologies against *S. aureus*.

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