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A Phase I, dose-escalation trial in adults of three recombinant attenuated *Salmonella* Typhi vaccine vectors producing *Streptococcus pneumoniae* surface protein antigen PspA

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ABSTRACT

Background: Live, attenuated, orally-administered *Salmonella* strains are excellent vectors for vaccine antigens and are attractive as vaccines based on previous use of *S.* Typhimurium in animals. A Phase I dose escalation trial was conducted to evaluate the safety and immunogenicity of three newly constructed recombinant attenuated *Salmonella enterica* serovar Typhi vaccine (RASV) vectors synthesizing *Streptococcus pneumoniae* surface protein A (PspA).
Methods: The 3 *S.* Typhi strains used as vectors to deliver PspA were *S.* Typhi ISP1820; *S.* Typhi Ty2 RpoS^{-}; and *S.* Typhi Ty2 RpoS^{+}. Sixty healthy adults (median age 25.2 years) were enrolled into 4 Arms (total 15 subjects per Arm); within each Arm, subjects were randomized 1:1:1 into 3 Groups of 5. All subjects in the same Group received the same vaccine vector, and all subjects in the same Arm received the same titer of vaccine (10^7, 10^8, 10^9 or 10^{10} CFU). Adverse events, safety, shedding, and IgG and IgA titers against *Salmonella* outer membrane proteins (OMPs), lipopolysaccharide (LPS) and PspA were evaluated.
Results: In the highest dose group, no subject experienced severe reactions or serious adverse events. Most adverse events were mild; one subject had a positive blood culture. No subject shed vaccine in stool. No statistically significant differences for post vaccination ELISA or ELISPOT results between Groups were detected. However, a limited number of ≥4 fold increases from baseline for IgA anti-OMPs, IgA and IgG anti-LPS, and IgA anti-PspA occurred for a few individuals as measured by ELISA, and IgA anti-OMPs as measured by ELISPOT assay.
Conclusions: All three *S.* Typhi vectored pneumococcal vaccines were safe and well-tolerated. Immunogenicity was limited possibly due to pre-existing high antibody titers prior to vaccination. Increases in IgA were most often observed.

1. Introduction

The use of orally administered, live, attenuated *Salmonella* strains presents an attractive alternative to killed and subunit vaccines for disease prevention. Attenuated *Salmonella* strains are excellent carriers for vaccine antigens, capable of stimulating strong systemic and local immune responses against synthesized recombinant antigens [1]. Despite success with recombinant attenuated *Salmonella enterica* serovar Typhimurium vectors in animals, recombinant attenuated *S. enterica* serovar Typhi vaccine (RASV) vectors expressing heterologous antigens have not been sufficiently effective in human studies to justify commercial development [2].

The poor immune responses produced by attenuated *S.* Typhi vectors in humans may be the result of hyperattenuation resulting from the mutations necessary for safety. However, it is also possible that the common *S.* Typhi parent of these vectors, Ty2, possesses a *rpoS* mutation that reduces immunity [3,4]. *RpoS* mutations attenuate *S.* Typhimurium [5], and the role of RpoS in

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Table 1
Vaccination schedule.

	Arms (15 subjects/Arm) and dose (CFU)		
	A	B	C
Groups 5 subjects/group Vaccine	χ9639(pYA4088) Ty2 RpoS$^-$ + PspA	χ9640(pYA4088) Ty2 RpoS$^+$ + PspA	χ9633(pYA4088) ISP1820 + PspA
Arm 1	10^7 CFU	10^7 CFU	10^7 CFU
Arm 2	10^8 CFU	10^8 CFU	10^8 CFU
Arm 3	10^9 CFU	10^9 CFU	10^9 CFU
Arm 4	10^{10} CFU	10^{10} CFU	10^{10} CFU

regulating genes on the *S.* Typhimurium virulence plasmid was demonstrated [6,7]. *S.* Typhimurium strains with *rpoS* mutations have markedly diminished abilities to colonize Peyer's patches in mice [8]. More recently [9,10,unpublished] Curtiss found that RpoS$^-$ *S.* Typhimurium mutants orally administered to mice are less immunogenic than RpoS$^+$ *S.* Typhimurium in inducing protective immunity to virulent *S.* Typhimurium challenge and in inducing immune responses to recombinant antigens. We theorized that *rpoS* mutations not only attenuate *S.* Typhi, but possibly lessen its immunogenicity.

Streptococcus pneumoniae is a leading cause of mortality in young children [9]. High manufacturing costs and increases in the rates of disease caused by pneumococci expressing capsules not covered by current conjugated polysaccharide vaccines make creating an inexpensive vaccine based on conserved pneumococcal protein antigens a high priority. The target antigen of such a vaccine must be a pneumococcal protein that contributes to virulence and is common to all serotypes and can elicit secretory and serum antibody responses via the mucosal route, resulting in protection against carriage as well as systemic disease [10–13]. Pneumococcal surface protein A (PspA)[14], a pneumococcal protein known to elicit protective immunity, was selected as the target antigen because RASVs synthesizing PspA have been successfully constructed and demonstrated to induce protective immunity in mice challenged with virulent *S. pneumoniae* [15–19].

This study evaluated the role of three newly constructed RASV strains synthesizing PspA in eliciting immunity to the heterologous antigen [20].

2. Methods

2.1. Study design

This Phase I dose escalation trial evaluated the safety and immunogenicity of three live-attenuated *S.* Typhi strains used as vectors to deliver PspA. The study was approved by the SLU Institutional Review Board and all subjects provided informed consent. The trial was divided into four Arms (1–4). Sixty adults were randomized 1:1:1 into three Groups (*S.* Typhi ISP1820, *S.* Typhi Ty2 RpoS$^-$, and *S.* Typhi Ty2 RpoS$^+$) of 5 subjects per Group in each of 4 Arms (total 15 subjects per Arm) (Table 1). All subjects in the same Group received the same vaccine and all subjects in the same Arm received the same dose of vaccine (10^7, 10^8, 10^9 or 10^{10} CFU). Dose escalation to the next Arm was dependent upon demonstrating the safety of the lower dose through Day 28. Subjects and staff were blinded to the Group assignment within each Arm. Subjects were confined for 11–15 days after vaccination. AdvantageEDC^{SM}, the EMMES Corporation's Internet Data Entry System, was used to randomize subjects.

2.2. Safety

Subjects had safety laboratory analyses (liver and kidney function and complete blood count) prior to vaccination and 2 weeks and 6 months post vaccination, and had a physical exam at admission and discharge from the confinement facility.

Safety evaluations included collection of systemic reactogenicity symptoms and character and frequency of stool for 15 days following each vaccination (Days 0–14) using memory aids, unsolicited adverse events through Day 28, and serious adverse events (SAE) throughout the study period.

Blood was collected twice daily for a minimum of seven days and stools or rectal swabs were collected once daily for a minimum of five days after vaccination for cultures to evaluate bacteremia and shedding, respectively. Blood was obtained at various time points for immunogenicity assays.

2.3. Subjects

Subjects, 18–40 years of age, were eligible for enrollment if they were healthy based on physical examination, medical history and laboratory assessment. Subjects were required to have a normal gallbladder ultrasound without cholelithiasis, normal and regular bowel habits with less than once per month use of laxatives or antidiarrheal agents.

Subjects were excluded if they had a previous *Salmonella* infection or pneumococcal vaccination; used H_2 blockers more than once a week; or had a diarrheal illness within 30 days prior to enrollment or allergy to specific antibiotics.

2.4. Vaccines

Three RASV vaccines synthesizing *S. pneumoniae* PspA-RX1 antigen [χ9633(pYA4088) *S.* Typhi ISP1820, χ9639(pYA4088) *S.* Typhi Ty2 RpoS$^-$ and χ9640(pYA4088) *S.* Typhi Ty2 RpoS$^+$] were provided by Dr. Roy Curtiss 3rd [20]. These strains exhibit arabinose-dependent regulated delayed attenuation. Thus, at the time of immunization, the vaccine displayed wild-type like attributes, but the vaccine rapidly attenuated in vivo due to the absence of arabinose [21]. Similarly, antigen synthesis was regulated by the presence of LacI (itself under the control of arabinose), causing PspA production to be delayed until after invasion of the host intestinal tissues [22]. Master and working seed stocks of the RASV strains were maintained at −70 °C in a phytone-glycerol solution. KT broth, a proprietary animal-free complex medium similar to Terrific Broth [23], was used for rapid and high-density growth of *S.* Typhi vaccine strains.

2.5. Preparation and administration of live vaccine doses

Preparation of the live vaccine doses began with cultures started from working seed stocks and incubated at 37 °C on the night prior to vaccine administration. The following morning, they were subcultured in fresh KT broth, and incubated at 37 °C with aeration. Growth of cultures was monitored periodically by spectrophotometer and harvested when target optical densities at 600 nm representing culture densities of 1×10^9 colony forming units per milliliter (CFU/mL) were reached. Cultures were pelleted and resuspended in sterile phosphate buffered saline to provide concentrated homogenous suspensions containing 1×10^{10} CFU/mL, which were diluted in sterile PBS as needed to prepare CFU doses specified for each study Arm in a final volume of 10 mL/dose. Gram stain, antigen-specific antiserum agglutination, and OD_{600} spectrophotometry of cultures suspended in PBS were used to verify purity, identity and concentration of vaccine products. Vaccine doses were administered to volunteers within 1 h of preparation.