Abstract

Title of Thesis: "**Designing broad-specificity, multi-subunit vaccines against enteric infections and studies on their immunogenicity and protective efficacy in animal models**",

Submitted by: **Risha Haldar**, Index No.: **75/22/Life Sc./27**

Enteric fever poses global public health challenges due to low diagnostic yield, antibiotic resistance, and asymptomatic carrier states. Limited long-term efficacy of current typhoid vaccines, especially in smaller children, and non-availability of vaccines against other *Salmonella* serovars necessitate the development of a multivalent vaccine with wider coverage against *Salmonella* serovars. Currently licensed typhoidal vaccines include Vi-TT, Vi PS, and Ty21a, while *S.* Paratyphi, *S.* Typhimurium and *S.* Enteritidis vaccines are in clinical trials. Multivalent vaccines, such as Vi-protein conjugates co-formulated with OSP-protein conjugates are also under development. The development of multiple combined glycoconjugates is time-consuming and expensive. Here, we have developed glycoconjugates, comprising of O-specific polysaccharide (OSP) from *Salmonella* Typhimurium or Vi polysaccharide of *Citrobacter fruendii*, conjugated to outer membrane protein T2544 of *Salmonella* Typhi/ Paratyphi to provide a single vaccine formulation for typhoidal and non-typhoidal *Salmonella* serovars. We had earlier reported the immunogenicity and protective efficacy of recombinant T2544 of *Salmonella* Typhi in a mouse model, promoting robust induction of serum IgG, intestinal secretory IgA, and *Salmonella*-specific T cells and memory responses. BALB/c and C57BL/6 mice were immunized subcutaneously with the OSP-rT2544 on days 0, 14, and 28. Immunized mice were protected against *S.* Typhi, *S.* Paratyphi A and *S.* Typhimurium and cross-protected against *S.* Enteritidis. OSP-rT2544 immunization augmented serum IgG and intestinal sIgA responses, along with strong antibody recall response with higher avidity serum IgG against both OSP and T2544. Significantly raised SBA titers of both primary and recall antibodies were observed against different *Salmonella* serovars. Bacterial motility was inhibited by secretory antibodies, supporting their role in vaccine-induced protection. Finally, robust induction of T effector memory response indicates long-term efficacy of the candidate vaccine. For Vi-conjugate vaccine studies, BALB/c mice were immunized subcutaneously and intramuscularly with a single dose of Vi-rT2544 and its immunogenicity and protective efficacy were compared with Vi-TT. Vi-T2544 immunized mice protected against both *S.* Typhi and *S.* Paratyphi A, while Vi-TT was effective against *S.* Typhi alone. The candidate Vi-rT2544 vaccine elicited high titers of functional serum IgG antibodies and memory T and B cell response, underscored by the induction of higher avidity and titers of recall antibodies following a booster. Besides *Salmonella*, enteric infection of *Shigella* poses significant public health challenge in the developing world. However, lack of a widely available mouse model recapitulating human shigellosis necessitate the establishment of newer model for better understanding of disease pathogenesis and development of drugs and vaccines. To develop a *Shigella* infection model, BALB/c mice were pre-treated with streptomycin and iron ($FeCl_3$) plus desferrioxamine (chelator) intraperitoneally, followed by oral infection with *Shigella* spp. Oral challenge of mice with virulent *S. flexneri 2a* resulted in diarrhoea, body weight loss, bacterial colonization and progressive colitis with raised proinflammatory cytokines and chemokines in the large intestine. To determine if the new oral shigellosis model was useful for vaccine efficacy studies, we generated a subunit vaccine based on a recombinant protein (IpaB) from *Shigella* and delivered it intranasally. Immunized mice conferred protection against different *Shigella* serovars. Further, to protect both *Shigella* and typhoidal *Salmonella*, rIpaB was fused with rT2544 to develop a chimeric vaccine, rIpaB-T2544. Vaccinated mice mounted antigen-specific, serum IgG and IgA antibodies and a balanced Th1/Th2 response and were protected against oral challenge with *S. flexneri 2a*, *Salmonella* Typhi and *Salmonella* Paratyphi A. Overall, our vaccine candidates may be a game changer by preventing multiple enteric infections simultaneously.

Santa Sabuj Das

सान्तासबुज दास, एस. डि.
SANTASABUJ DAS, M.D.
वैज्ञानिक (एफ) / Scientist (F)
आइ.सि.एम.आर. राष्ट्रीय कॉलरा और आंत्र रोग संस्थान
ICMR National Insurance of Cholera & Enteric Diseases
पी-३३,सी. आई. टी. रोड, स्कीम-७०एम, बेलियाघाटा, कोल-१०
P-33, CIT Road, Scheme-XM, Beliaghata, Kolkata-10
E-mail : santasabujdas@yahoo.com

Risha Haldar
18.11.2024