IgG and IgA antigen-specific B memory responses in healthy U.S. adults immunized with a parenteral Trivalent Salmonella Conjugate Vaccine (TSCV)

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Trivalent *Salmonella* Conjugate Vaccine (TSCV) Design

- **S. Enteritidis-** and **S. Typhimurium-Core-O-polysaccharide (COPS)** were conjugated with the corresponding serovar specific unpolymerized flagellin subunits (FliC).

- **S. Typhi Vi-antigen** conjugated with Tetanus Toxoid (Typbar-TCV®) vaccine against Typhoid Fever was manufactured by Bharat Biotech, Hyderabad, India.

- **TSCV** is designed to prevent infections caused by both Typhoidal (**S. Typhi**) and invasive Non-Typhoidal *Salmonella* (iNTS), especially in younger population.
Double-blinded, randomized, placebo-controlled, dose-escalation study (CVD1000)

**Cohort A:** Single IM dose of 6.25 µg (¼ of the highest vaccine dose)
Vaccinees N=8 ; Placebo N=2

**Cohort B:** Single IM dose of 12.5 µg (½ of the highest vaccine dose)
Vaccinees N=10 ; Placebo N=2

Initially planned highest single vaccine dose (Cohort C: 25 µg, Single IM) as well as a two-dose regimen (Cohort D) studies which could not be implemented due to the COVID pandemic

However, a modified phase 1b study (CVD 2000) evaluating higher doses of TSCV vaccine was conducted and completed 2022-2023
Induction of Antibody Secreting cells (ASC) & Memory B cells (B_M)

Vaccination

Ag

ASC

Germinal Center

Lymph node

BN

B_M

Blood

ASC

Recall

Recirculate

Tissue Homing (Marker)

Lymph Node (CD62L)

Bone Marrow

Gut Mucosa (Integrin-\(\alpha4\beta7\))

Other Sites (e.g., Liver)

Naïve B cells (BN)

Antibody Secreting Cells (ASC)

Apoptotic ASC

Memory B cells (B_M)

ASC peak
7-10 days post-exposure

7-10 days post-exposure
Methodology: ASC and $B_M$ Measurement

1. ASC measurements were performed on freshly isolated PBMC on Pre-vaccination D1 and Post vaccination D8 (only on cohort B)

2. $B_M$ assays were performed with *in vitro* (5 days) expanded B cells using cryopreserved PBMC obtained on D1 (baseline), D29, D57 & D510/410 (cohorts A & B)

3. B cell ELISPOT assays were performed by seeding (Fresh or Expanded) cells into antigen-coated mixed cellulose membrane plate wells

4. Antigens Used
   
   I. Vi antigen (Vi)
   
   II. LPS: S. Enteritidis (SE), S. Typhimurium (STm), S. Cholerasuis (Negative control)
   
   III. SE & STm Flagellin (FliC)
   
   IV. Tetanus Toxoid (TT)
   
   V. Total IgG and IgA (Positive Control)
   
   VI. Media only (No antigen - Negative controls)
Induction of antigen specific ASCs 7 days following TSCV immunization (Cohort B: 12.5 µg group)

Data presented are increases from pre-vaccination levels (Day 1)
Horizontal and Error bar (Mean +/- 1SE)

P value *<0.05, **<0.01 by Wilcoxon Matched Paired Rank Test
comparing the corresponding IgG vs IgA levels

Vi: S. Typhi Vi antigen
SE: S. Enteritidis LPS
STm: S. Typhimurium LPS
FliCs: Flagellin (SE&STm)
TT: Tetanus Toxoid

Circles: IgG
Squares: IgA

Virtually No ASC responses were observed in Placebos (n=2)

Visit Poster #118 on Thursday for details on ASC & Homing studies
Induction of antigen specific IgA $B_M$ against polysaccharide components among vaccinees
Induction of antigen specific IgG $B_M$ against polysaccharide components among vaccinees

% of Total IgG

Days after Immunization

Cohort A Vi IgG %

Cohort A LPS SE IgG %

Cohort A LPS STm IgG %

Cohort B Vi IgG %

Cohort B LPS SE IgG %

Cohort B LPS STm IgG %

% of Total IgG

Days after Immunization

6.25 µg

12.5 µg
Induction of Polysaccharide Antigen specific IgA B<sub>M</sub> among vaccinees from both A & B Cohorts (N=18)

A. Vi IgA

B. LPS SE IgA

C. LPS STm IgA

Days after Immunization

% of Total IgA

P value *<0.05, **<0.01 ***<0.001: Wilcoxon Matched Paired Rank Test
Induction of Polysaccharide Antigen specific IgG B<sub>M</sub> among Vaccinees from both Cohort A & B (N=18)

A. Vi IgG

B. LPS SE IgG

C. LPS STm IgG

Days after Immunization

P value *<0.05, **<0.01 ***<0.001: Wilcoxon Matched Paired Rank Test
Percentage of vaccine responders to polysaccharide Antigen-specific IgG and/or IgA BM in all vaccinees

Responders: Volunteers showing Post-vaccination increases (Post-vaccination-pre-vaccination) of ≥0.1% in IgG or IgA B Memory (as % of corresponding total IgG or IgA) at any of the post-vaccination days measured. Data are shown as % of responders among the volunteers from both cohorts A & B (n=18).

* p<0.05  -  IgA compared to the corresponding IgG. Chi square Test
Percentage of vaccine responders to conjugate proteins
Antigen-specific IgG and/or IgA $B_M$ in all vaccinees

Responders: Volunteers showing Post-vaccination increases (Post-vaccination-pre-vaccination) of $\geq 0.1\%$ in IgG or IgA $B_M$ Memory (as % of corresponding total IgG or IgA) at any of the post-vaccination days measured. Data are shown as % of responders among the volunteers from both cohorts A & B (n=18).

* $p<0.05$ - IgG compared to the corresponding IgA. Chi square Test
Summary (I)

- Single dose intramuscular immunization with ¼ (6.25 µg) and ½ (12.5 µg) strength doses of the intendent full dose (25 µg) of TSCV was found to be highly immunogenic (ASC and homing data: Poster #118).

- We observed induction of both IgG or IgA BM responses to polysaccharide antigens (e.g., Vi, LPS SE and LPS STm) that peaked at D29 post-vaccination; however, in some cases instances remained detectable on D57 and later (days 510 or 410 post-vaccination)

- A significant percentage of volunteers showed Post-vaccination increases in both IgG and/or IgA responses against Vi (72%), LPS STm (78%) and LPS SE (61); but not (1 in 17: 6%) against an unrelated LPS purified from S. Cholerasuis (negative control - data not shown).
As expected, a re-call response to TT was observed in 76% of the participants, which was exclusively mediated of IgG B_M. Interestingly, albeit in lower percentages, we observed induction of both Ig and IgA B_M responses to FliC STm and Flic SE conjugate proteins in 56% and 41% of the participants, respectively.

These encouraging data show the induction of both IgG and IgA B_M cells, following IM TSCV immunization.

Ongoing assays with a larger number of participants (CVD 2000 study) will reveal if the B_M responses observed in this study could be further improved, particularly against FliCs, with the full dose (25 µg) immunization.
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