Assessing the protective efficacy of an Intranasal vaccine candidate, rCTB-T2544, against Typhoid and Paratyphoid infection using Iron overloaded murine model

Presenter-
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T2544, an outer membrane protein of *S. Typhi*, is involved in host cell Adhesion and Pathogenesis

- T2544 is required for adhesion of *S. Typhi* to the host cell.
- Mutant T2544 bacteria cannot cause typhoid.
- T2544 was reported to be highly immunogenic.
- T2544 specific IgG titers are detected in immunized mice.
- Immunized mice are protected against oral *S. Typhi* challenge.

Hence we hypothesize to deliver rT2544 through nasal mucosal route using mucosal adjuvant.
Cholera toxin subunit B (CTB) is the nontoxic portion of cholera toxin. It binds to the monosialotetrahexosylganglioside (GM1).

CTB administered through non-oral mucosal routes significantly enhanced antigen-specific humoral and cell-mediated immunity, not only at the local site, but also at distal mucosa, a phenomenon called ‘common mucosal immunity.’

Used as Non toxic mucosal adjuvant against Influenza virus, Helicobacter pylori, Streptococcus pneumoniae, Bordetella pertussis, and Francisella tularensis.
Objectives-

Construction of CTB-T2544 and assessing immunogenecity

Proposed iron overload model

Assessing the efficacy of an CTB-T2544 against S. Typhi and Paratyphi A infection
Construction of potential vaccine candidate rCTB-T2544

Schematic diagram

Clone confirmation digestion of CTB-T2544 in pET28a

Cholera toxin B 309 bp

T2544 with linker 663 bp

Clone confirmation PCR of CTB-T2544 in pET28a 972 bp
Purification and Functional characterization of the conjugate

Western blot

rCTB-T2544 in non reducing and reducing gel for confirming multimer

Pentameric rCTB-T2544 binds to GM1 in dose dependent manner
Immunization schedule

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intranasal</td>
<td>60ug/mouse CTB-T2544/ T2544/CTB</td>
<td>60ug/mouse CTB-T2544/ T2544/CTB</td>
<td>60ug/mouse CTB-T2544/ T2544/CTB</td>
<td>Oral infection</td>
<td></td>
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</tbody>
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Blood and fecal sample collection

Observation
Increased IgG, IgG1, IgG2a and IgA in the CTB-T2544 group
Substantial numbers of T2544-specific IgG and IgA antibody-secreting cells (ASCs) in the spleen, MLN and Peyer’s Patches (PP) after intranasal CTB-T2544.
Mucosal antibody-

A strong T2544-specific sIgA response in the fecal and intestinal secretions of the CTB-T2544 immunized mice
Functional validation –
1. Adhesion Inhibition assay

HT-29 cell ➔ Microscopy

GFP DIC HOECHST MERGE

Fe Pre immune
T2544
CTB-T2544

GFP DIC HOECHST MERGE

Int Pre immune
T2544
CTB-T2544

GFP DIC HOECHST MERGE

Se Pre immune
T2544
CTB-T2544
Functional Validation - Opsanophagocytosis

THP1 cell → Microscopy

**Fe**

- GFP
- DIC
- HOECHST
- MERGE

- Pre immune
- T2544
- CTB-T2544

**Int**

- GFP
- DIC
- HOECHST
- MERGE

- Pre immune
- T2544
- CTB-T2544

**Se**

- GFP
- DIC
- HOECHST
- MERGE

- Pre immune
- T2544
- CTB-T2544
Serum Cytokine profile

We found significantly elevated, circulating Th1 (IL12 and IFNγ) and Th2 (IL-4, IL-5) cytokines.

The number of IFNγ- and IL-17A-secreting T cells (Th1 and the Th17 cells) in the Peyer’s Patches was also increased after CTB-T2544 immunization.
Increased Follicular helper T cells post CTB-T22544 immunization-

TFH cell number in MLN after immunization with CTB-T2544 doubled compared with T2544 or no immunization (6.03% vs 3.06% vs 2.74%)
Objectives-

Construction of CTB-T2544 and assessing immunogenicity

Proposed iron overload model

Assessing the efficacy of an CTB-T2544 against S. Typhi and Paratyphi A infection
Iron overloaded mouse model -

Desferrioxamine (0.025 mg/g body weight) followed by Ferric chloride (0.32 mg/g body weight).

5 hr

Salmonella infection

Survival assay

An adhesion protein of *Salmonella enterica* serovar Typhi is required for pathogenesis and potential target for vaccine development

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Objectives-

Construction of CTB-T2544 and assessing immunogenicity

Proposed iron overload model

Assessing the efficacy of an CTB-T2544 against S. Typhi and Paratyphi A infection
Protection against *S*. Typhi-

- CTB-T2544: 70%
- T2544
- CTB
- PBS

Protection against *S*. Paratyphi A-

- Control
- T2544 (IN)
- CTB-T2544 (IN)

80%
Adoptive transfer and challenge experiment:

For immune serum – Adoptive transfer of serum followed by infection resulted in a 25% survival rate.

For mucosal antibodies- Pre-incubated S. Typhi with intestinal lavage and fecal extracts from the immunized mice for 30 minutes before infecting the naïve mice followed by infection killed only 50% mice, while with a sublethal dose showed significant reduction in the colonization of the intestine.
Conclusion

rCTB-T2544 Induces antigen specific Humoral and mucosal antibody response.

rCTB-T2544 Induces mixed Th1, Th2 and Th17 cytokines with follicular helper T cells creating a protective milieu in the intestine.

rCTB-T2544 immunization protects against S. Typhi and S. Paratyphi A infection