

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



Presenter-

Suparna Chakraborty

PhD student.

Supervisor- Dr. Santasabuj Das
ICMR-NICED, INDIA.

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 is protected against oral *S. Typhi* challenge

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

Shubhamoy Ghosh^a, Krishnendu Chakraborty^a, Theeya Nagaraja^a, Surajit Basak^b, Hemanta Koley^c, Shanta Dutta^c, Utpala Mitra^a, and Santasabuj Das^{a,b,1}

^aDivision of Clinical Medicine, ^bBiomedical Informatics Center, and ^cDivision of Bacteriology, National Institute of Cholera and Enteric Diseases, Kolkata 700010, India

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Vaccine

journal homepage: www.elsevier.com/locate/vaccine



A recombinant protein of *Salmonella* Typhi induces humoral and cell-mediated immune responses including memory responses

Sayan Das, Rimi Chowdhury, Shubhamoy Ghosh¹, Santasabuj Das^{*}

Division of Clinical Medicine, National Institute of Cholera and Enteric Diseases, Kolkata, India

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*

Differential Requirements for Protection against Mucosal Challenge with *Francisella tularensis* in the Presence versus Absence of Cholera Toxin B and Inactivated *F. tularensis*¹ **FREE**

Constantine Bitsaktsis, ... et. al

J Immunol (2009) 182 (8): 4899–4909.

<https://doi.org/10.4049/jimmunol.0803242>

Siderophore-based immunization strategy to inhibit growth of enteric pathogens

Martina Sassone-Corsi^{a,b,1}, Phoom Chairatana^{c,1}, Tengfei Zheng^c, Araceli Perez-Lopez^{a,b}, Robert A. Edwards^d, Michael D. George^{e,2}, Elizabeth M. Nolan^{c,3,4}, and Manuela Raffatellu^{a,b,3,4}

^aDepartment of Microbiology and Molecular Genetics, School of Medicine, University of California, Irvine, CA 92697; ^bInstitute for Immunology, University of California, Irvine, CA 92697; ^cDepartment of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; ^dDepartment of Pathology and Laboratory Medicine, University of California, Irvine, CA 92697; and ^eDepartment of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, CA 95616

Edited by Ralph R. Isberg, Howard Hughes Medical Institute/Tufts University School of Medicine, Boston, MA, and approved July 11, 2016 (received for review April 25, 2016)

Intranasal immunization with influenza antigens conjugated with cholera toxin subunit B stimulates broad spectrum immunity against influenza viruses

Junwei Li, Maria T Arévalo, Yanping Chen, Olivia Posadas, Jacob A Smith, and Mingtao Zeng

Research Article

Immunization with the Recombinant Cholera Toxin B Fused to Fimbria 2 Protein Protects against *Bordetella pertussis* Infection

Noelia Olivera,¹ Celina E. Castuma,¹ Daniela Hozbor,¹ María E. Gaillard,¹ Martín Rumbo,² and Ricardo M. Gómez¹

Objectives-

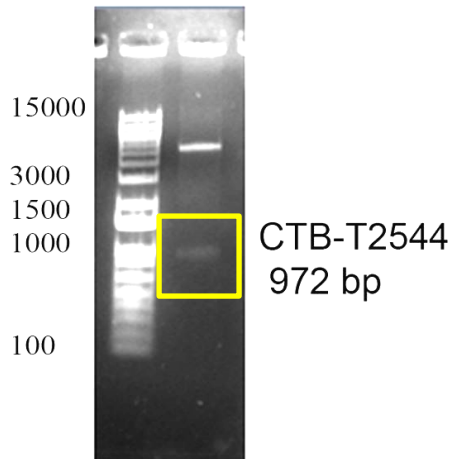
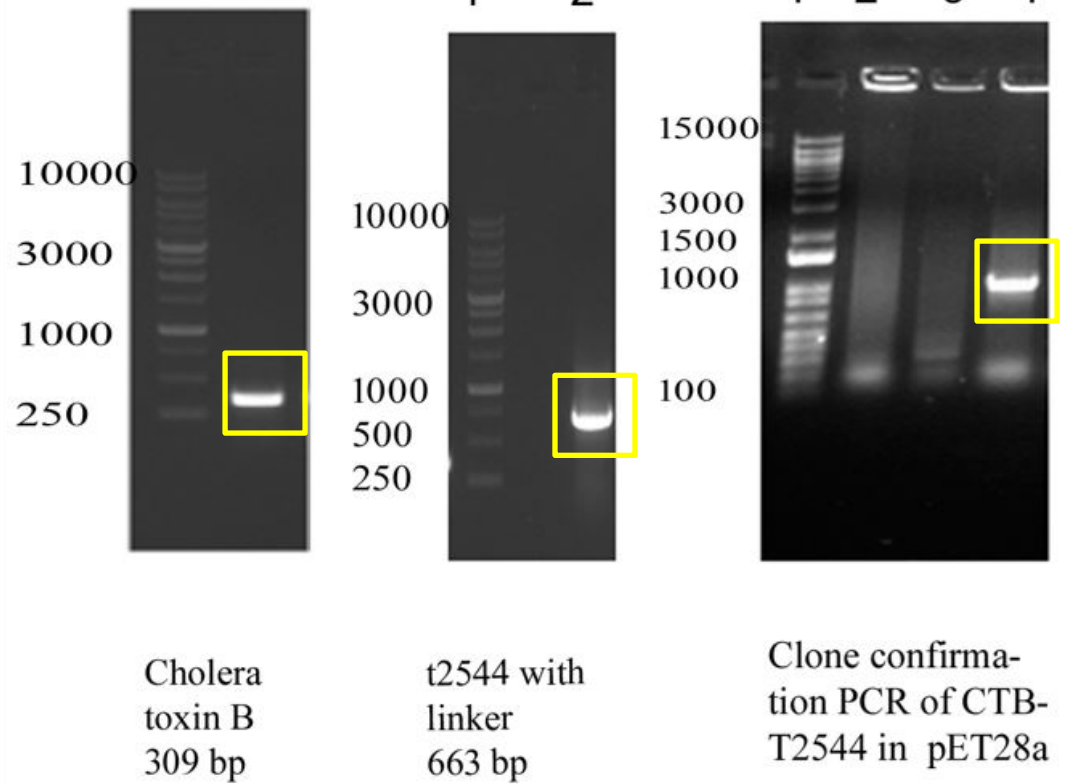
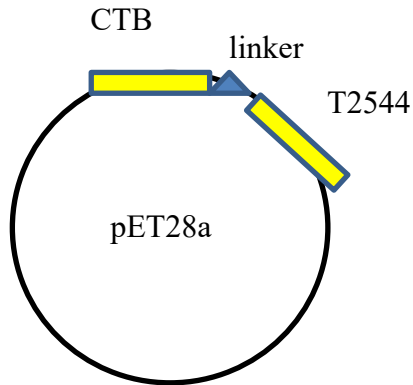
Construction of CTB-T2544 and assessing immunogenicity

Proposed iron overload model

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

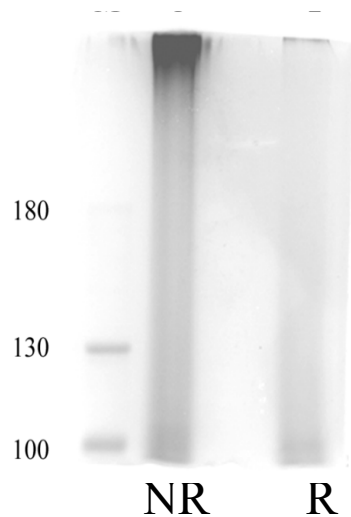
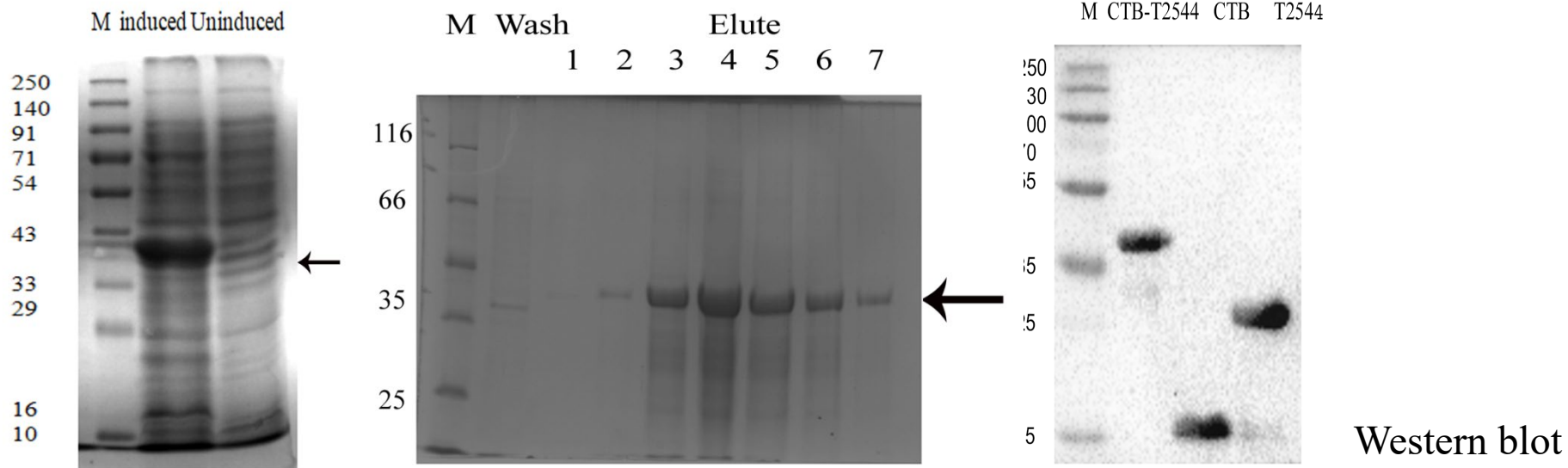
Construction of potential vaccine candidate rCTB-T2544

Schematic diagram

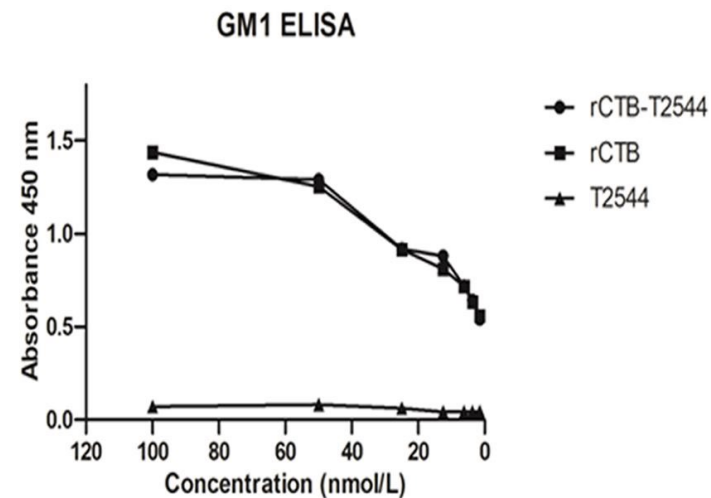


Clone confirmation digestion of CTB-T2544 in pET28a

Purification and Functional characterization of the conjugate

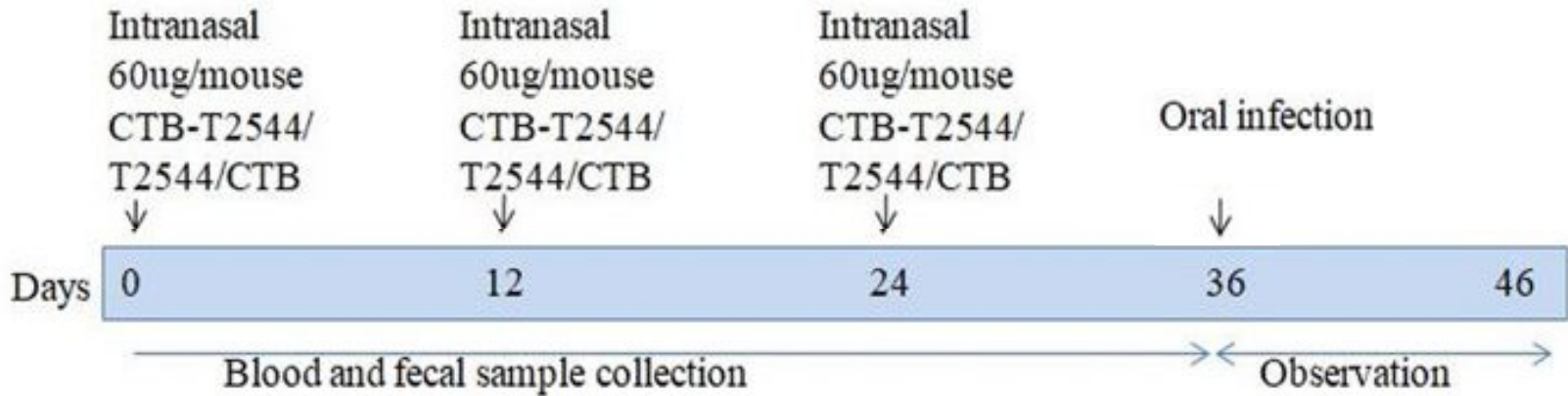
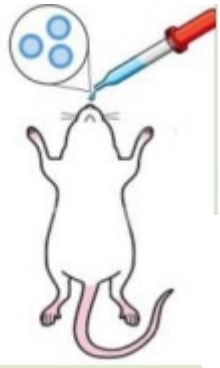


rCTB-T2544 in non reducing and reducing gel for confirming multimer

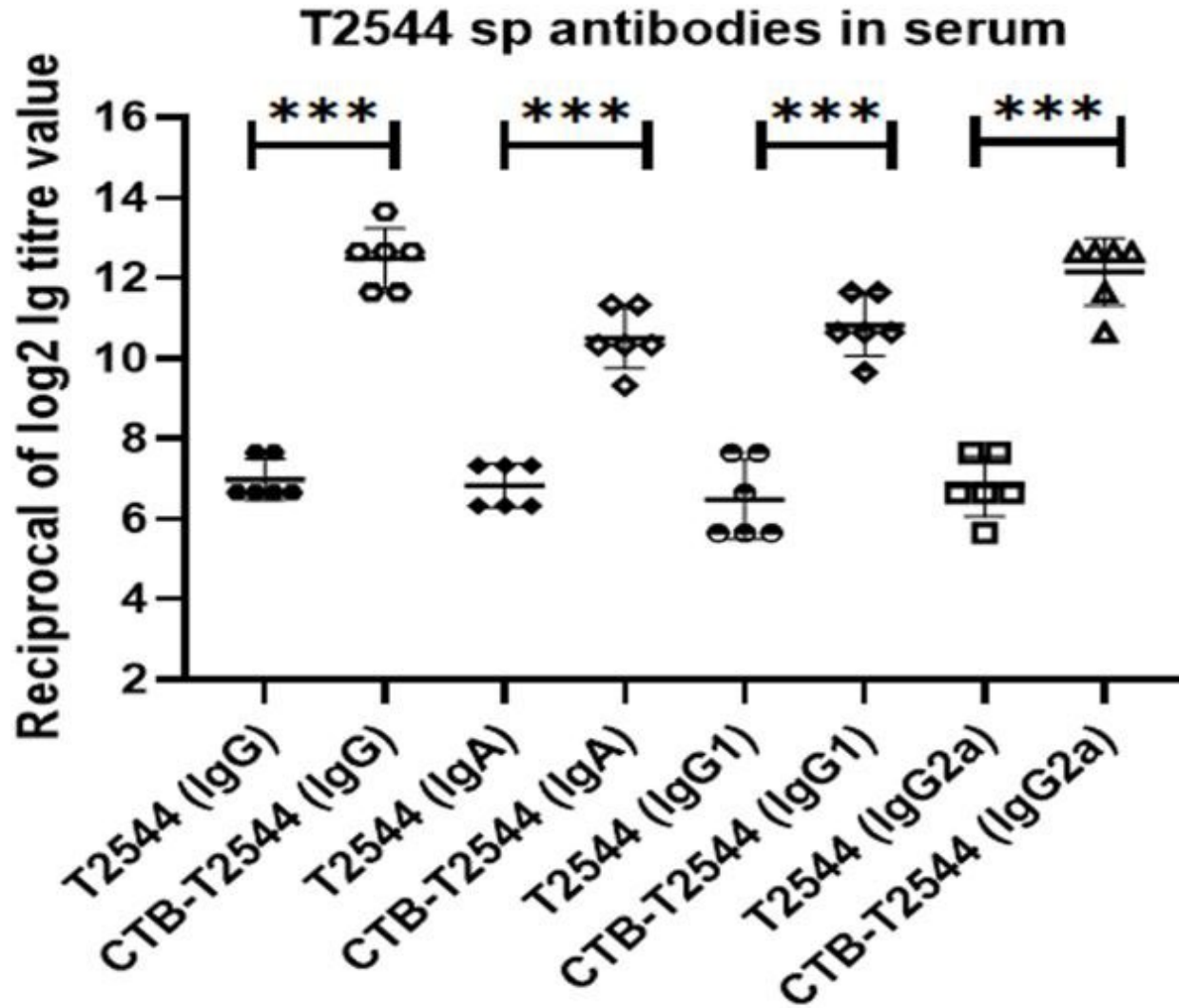


Pentameric rCTB-T2544 binds to GM1 in dose dependent manner

Immunization schedule

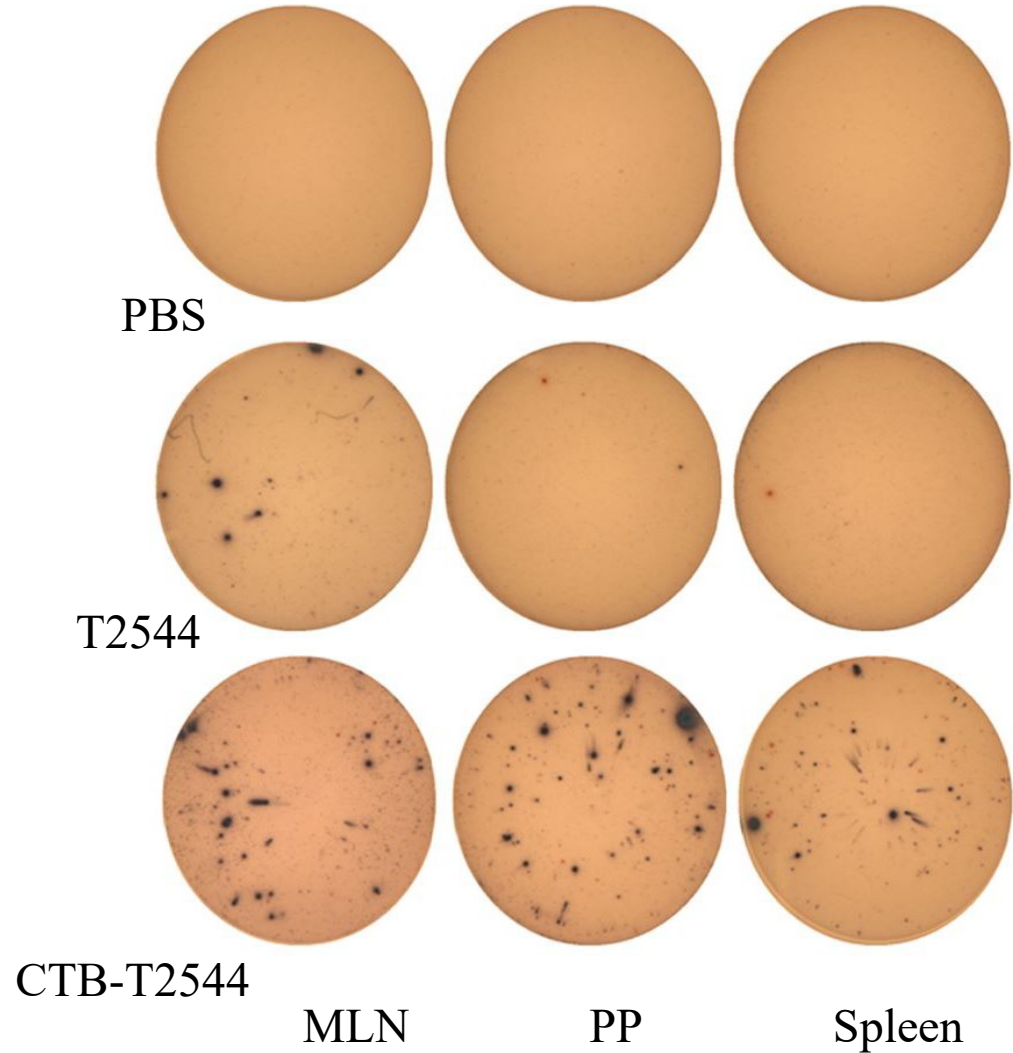
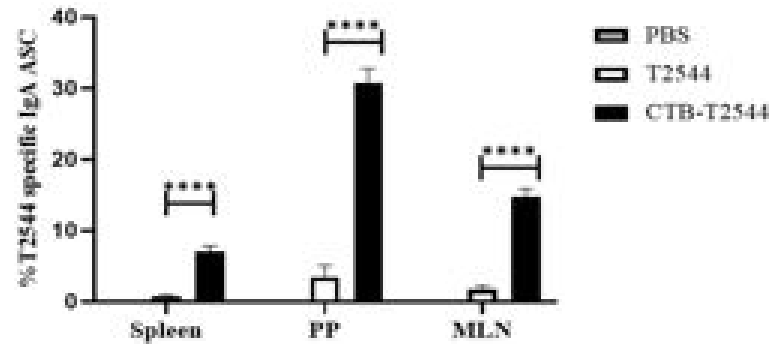
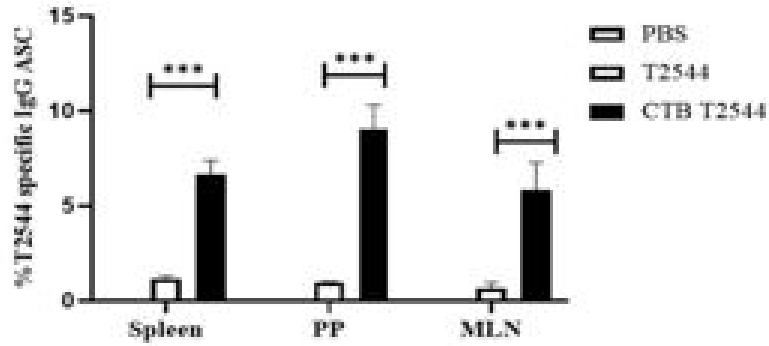


Humoral antibody-



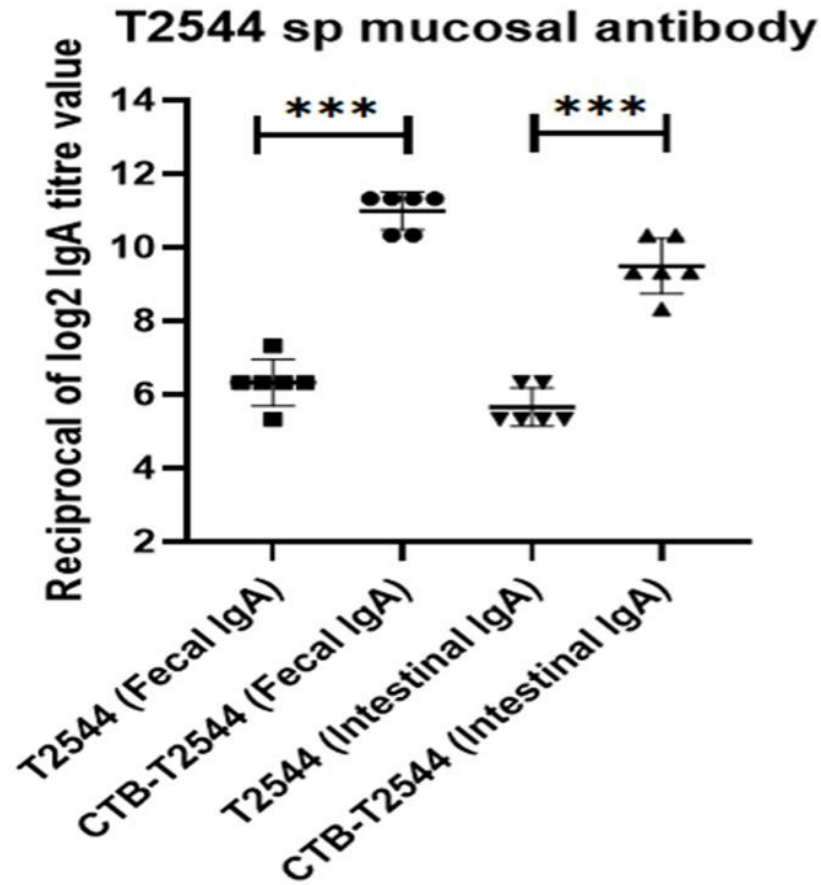
Increased IgG, IgG1, IgG2a and IgA in the CTB-T2544 group

T2544 sp antibody secreting cells



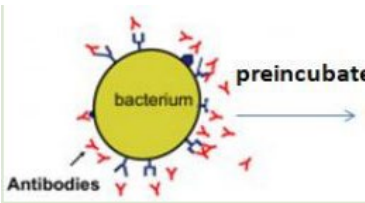
Substantial numbers of T2544-specific IgG and IgA antibody-secreting cells (ASCs) in the spleen, MLN and Peyer's Patches (PP) after intranasal CTB-T2544

Mucosal antibody-

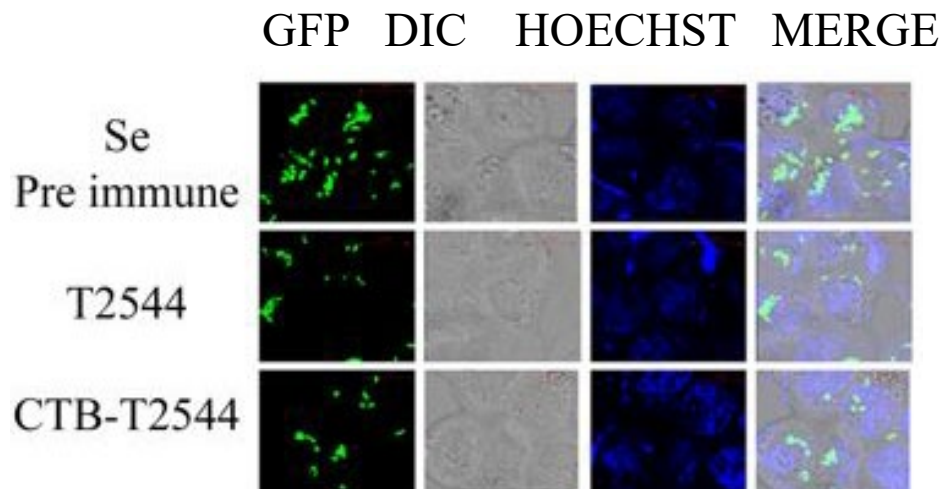
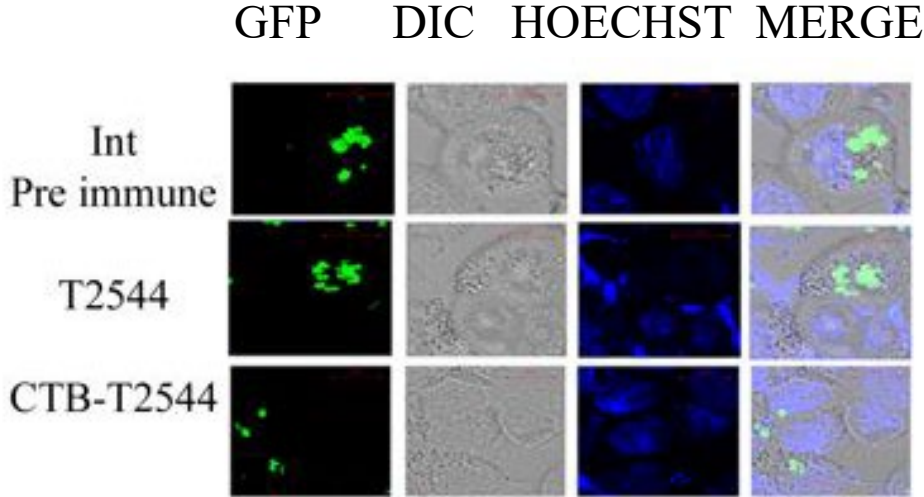
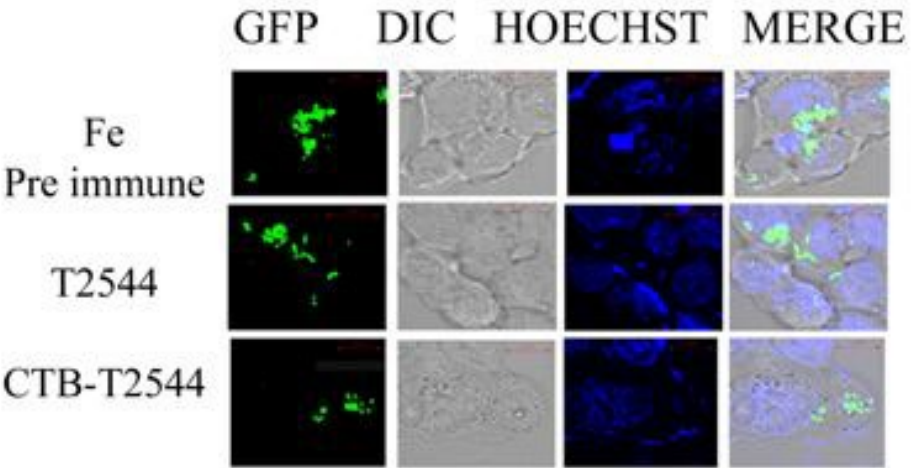


A strong T2544-specific sIgA response in the fecal and intestinal secretions of the CTB-T2544 immunized mice

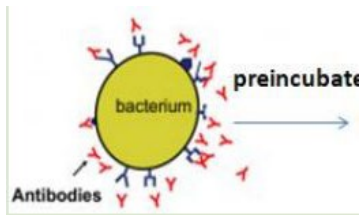
Functional validation - 1. Adhesion Inhibition assay



HT-29 cell → Microscopy



Functional Validation- Opsanophagocytosis

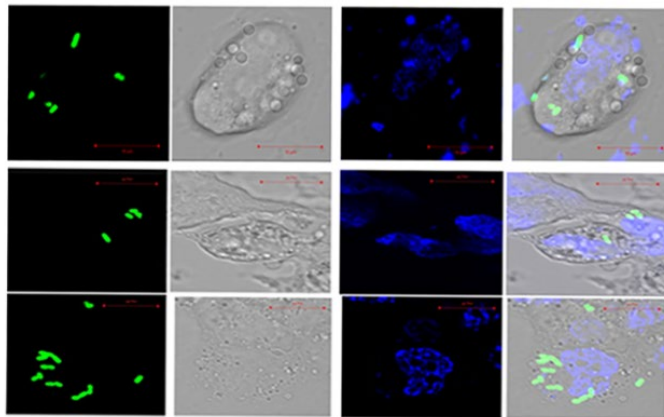


THP1 cell

Microscopy

Fe

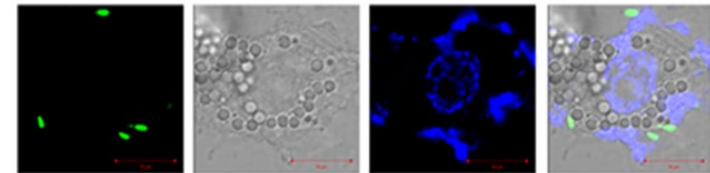
GFP DIC HOECHST MERGE



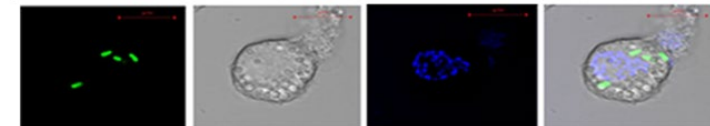
Int

GFP DIC HOECHST MERGE

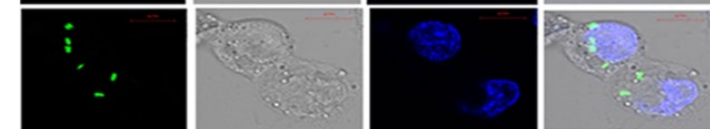
Pre immune



T2544



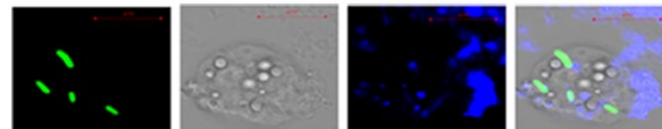
CTB-T2544



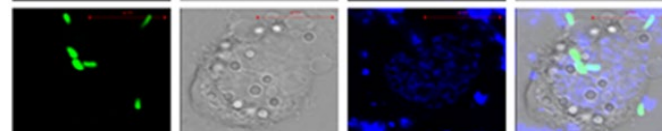
Se

GFP DIC HOECHST MERGE

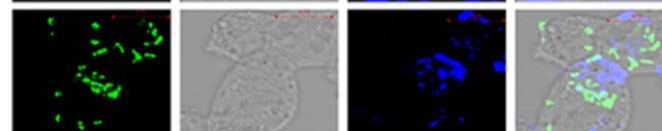
Pre immune



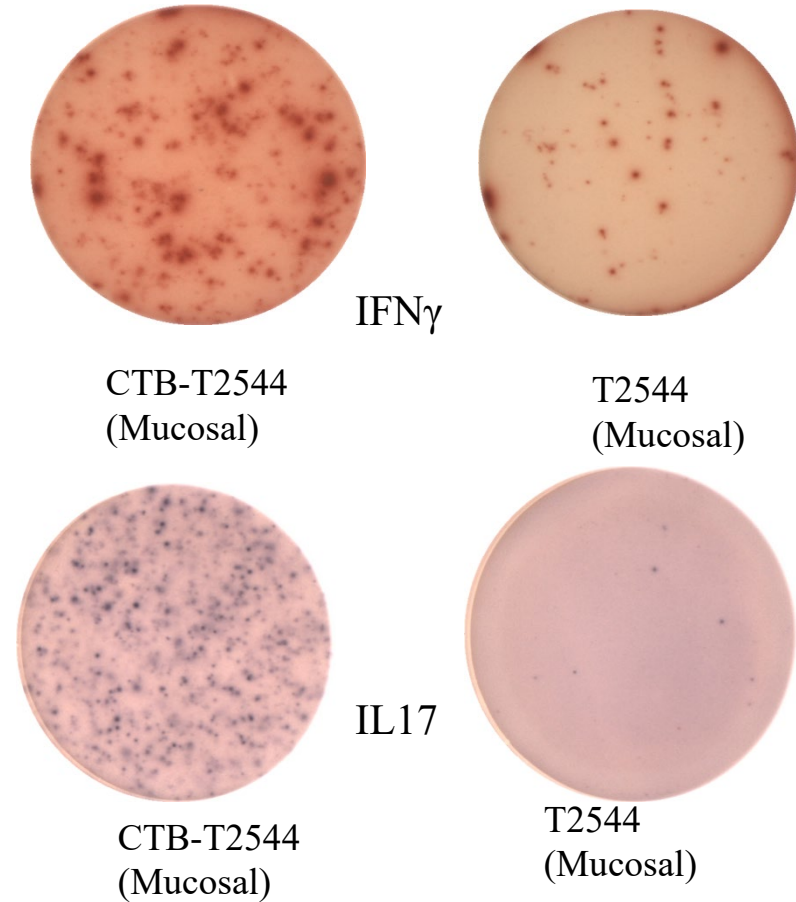
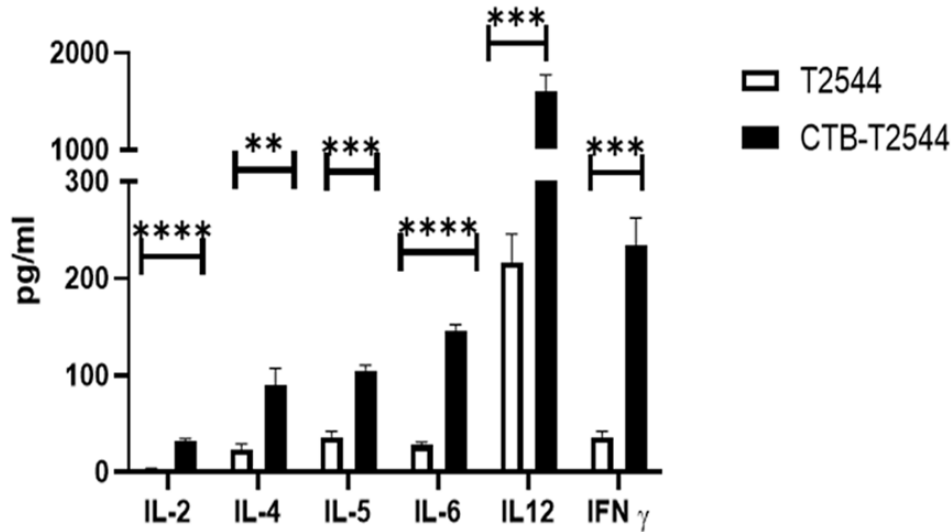
T2544



CTB-T2544



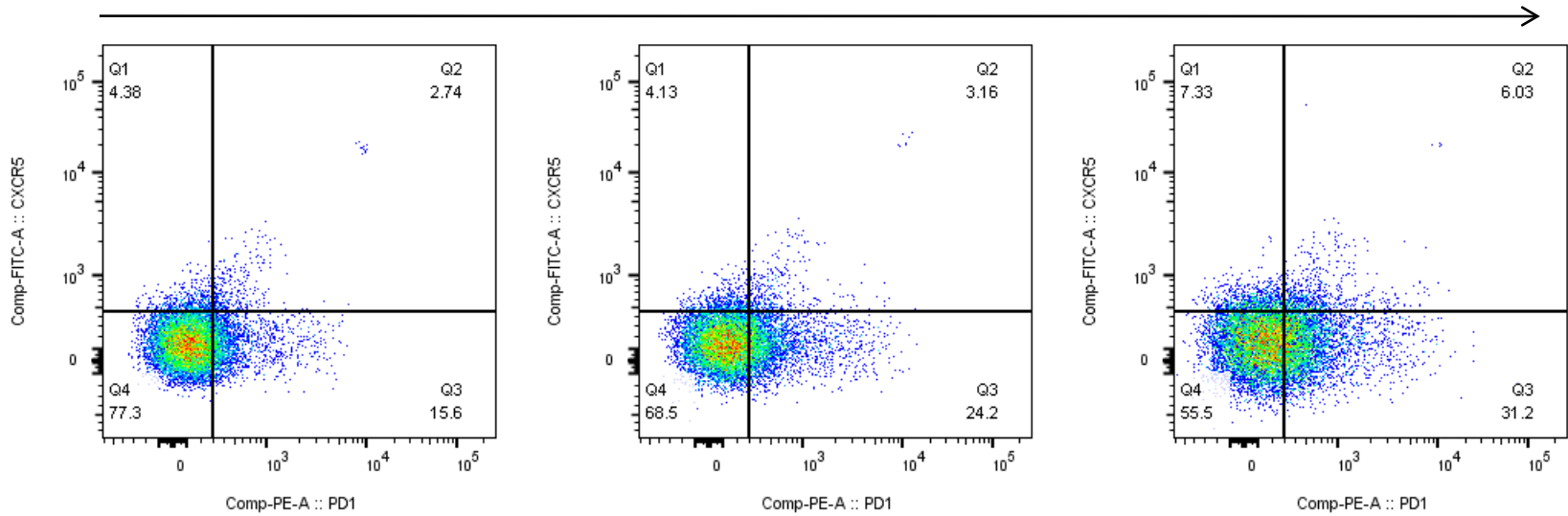
Serum Cytokine profile



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

The number of IFN γ - 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-

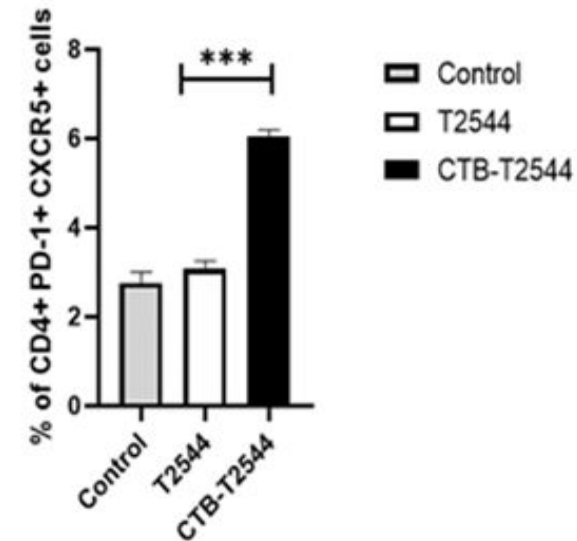


**Unimmunized
(2.74%)**

**T2544 IN immunized
(3.16%)**

**CTB-T2544 IN immunized
(6.03%)**

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

Shubhamoy Ghosh^a, Krishnendu Chakraborty^a, Theeya Nagaraja^a, Surajit Basak^b, Hemanta Koley^c, Shanta Dutta^c, Utpala Mitra^a, and Santasabuj Das^{a,b,1}

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PNAS

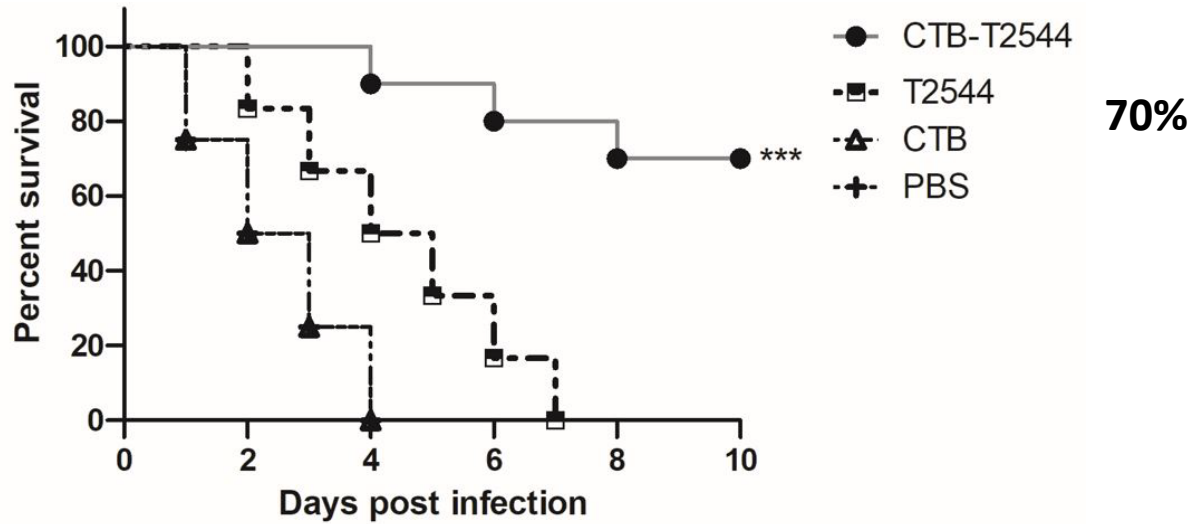
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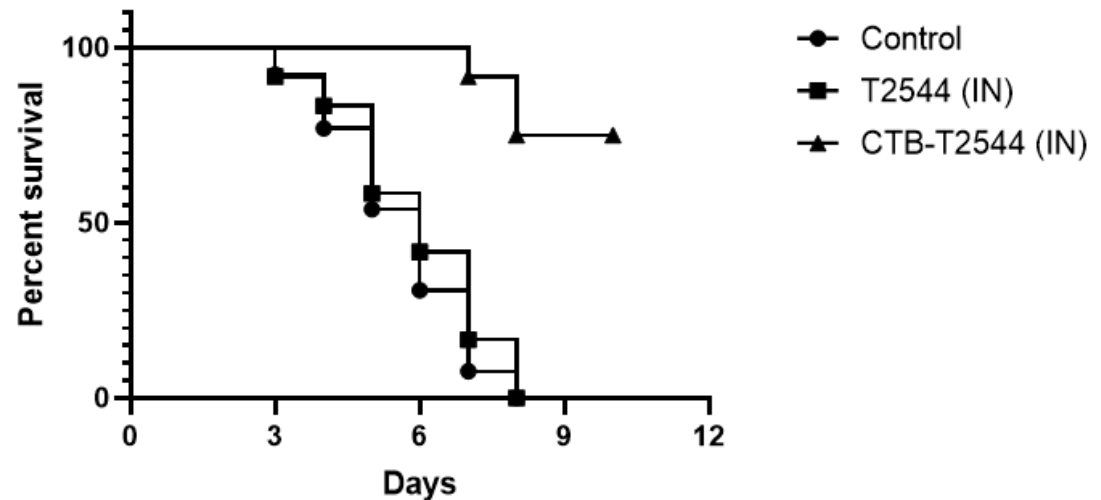
Proposed iron overload model

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

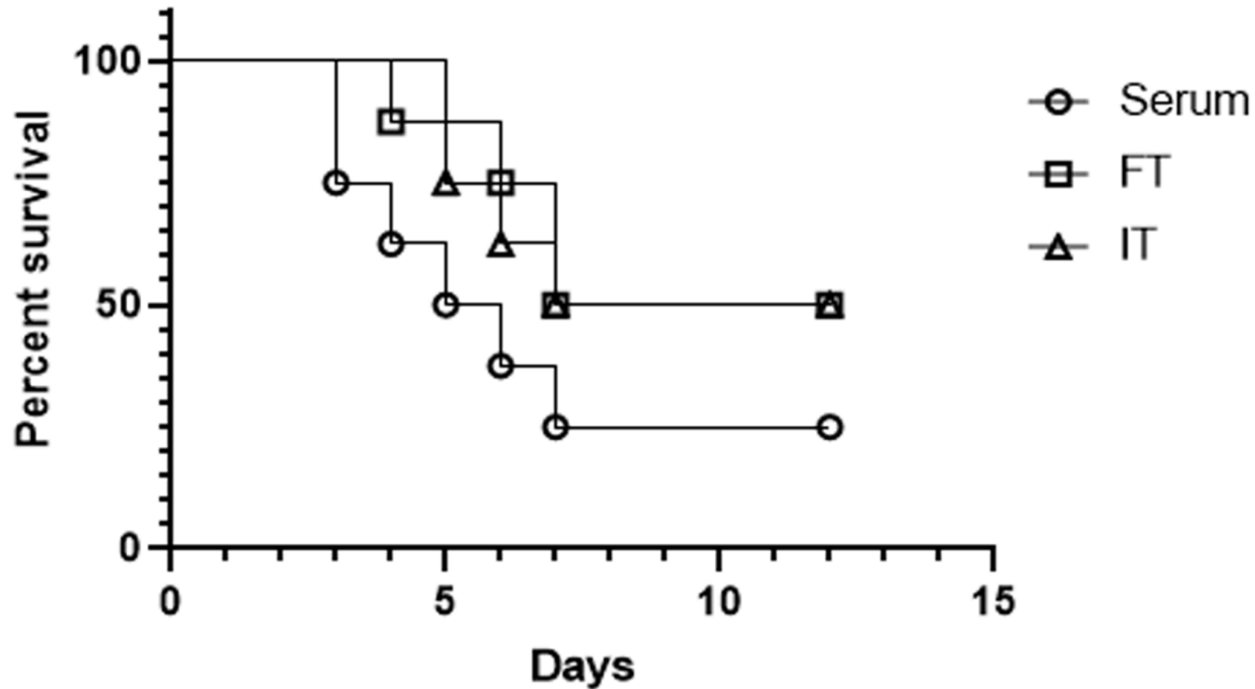
Protection against *S. Typhi*-



Protection against *S. Paratyphi A*-



Adoptive transfer and challenge experiment-



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

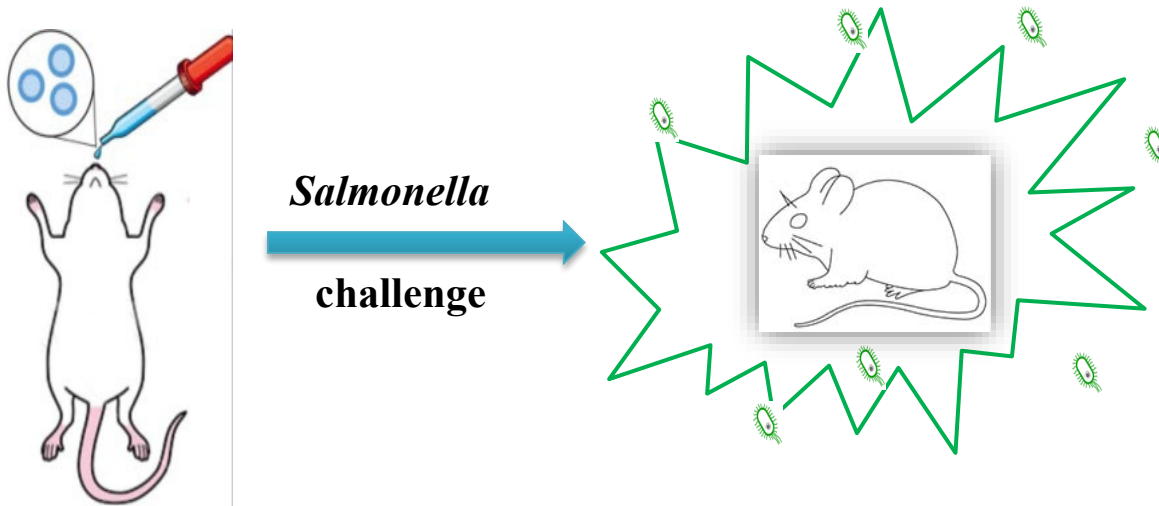
For mucosal antibodies- Pre-incubated *S. Typhi* with intestinal lavage and fecal extracts from the immunized mice for 30 minutes before infecting the naïve mice followed by infection killed only **50%** mice, while with a sublethal dose showed significant reduction in the colonization of the intestine.

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





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