Department of Biochemistry and Microbiology

Development of an orally administered nanoparticle vaccine to combat *Clostridioides difficile* infections

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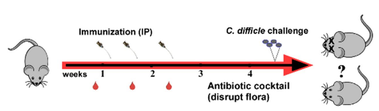
Abstract

Clostridioides difficile is a gram +, spore forming, toxin producing anaerobe that is found throughout the environment. *C. difficile* is the leading agent of hospital acquired infections. Symptoms of *C. difficile* infection can range from diarrhea to pseudomembranous colitis and if left untreated can lead to death. *C. difficile* is currently only treated with 3 antibiotics Metronidazole, Vancomycin, and Fidaxomicin. All these antibiotics are non-specific to *C. difficile* and have the side effect of killing the normal microbiome of the gut. This microbiome helps to keep the body resistant to *C. difficile* infections, and its destruction can lead to relapses of disease. Ongoing work in our lab is looking at preventing *C. difficile* infections using a nanoparticle based oral vaccine. In a mouse model of *C. difficile* infections, we previously demonstrated that intraperitoneal injection of a vaccine composed of the receptor-binding domain of *C. difficile* toxin B (TcdB) with chitosan and poly-g-glutamic acid were effective in inducing antigen-specific IgA and IgG antibodies. Continuing this research, we later demonstrated that rTcdB encapsulated in a polypeptide-based polymer and delivered orally produces a long lasting and robust antibody response. These robust antibody responses to the *C. difficile* toxin were enough to prevent disease within a mouse model, however, it was not able to reduce bacteria burden leaving the potential for asymptomatic spread and relapses of disease. In my project, I wish to continue this research in two ways. First, by continuing the optimization of the nanoparticle delivery system. To do this we propose using a nanoparticle polymer that specifically targets M-cells lining the intestine and be pH activated. We hypothesize that this will improve antigen immunogenicity and provide better protection against *C. difficile* infections. Second, evaluate several *C. difficile* surface proteins immunogenicity in a mouse model. We hypothesize that a two-target approach may decrease the bacterial load and lead to complete protection against *C. difficile* infections.

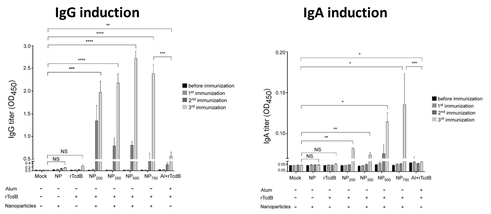
Study 1: Chitosan polymer vaccine (IP)

A. Nanoparticle vaccine design

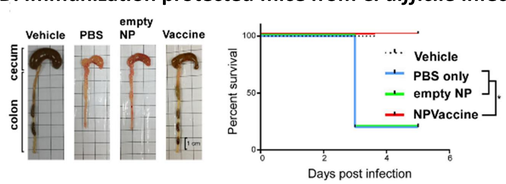
B. IP Vaccination regiment and infection protocol



C. Induction of antigen-specific antibodies



D. Immunization protected mice from *C. difficile* infection

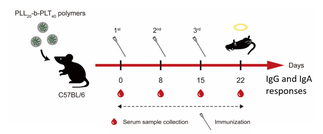


- Intraperitoneal (IP) injection of rTcdB-encapsulated nanoparticles induced specific antibodies
- 3-dose IP immunization protected mice from lethal challenge with *C. difficile* spores
- Results published: Liu et al. Front Microbiol. 2017 Jul 25;7:1411.

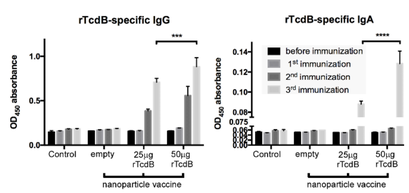
Study 2: Amino acid polymer vaccine (Oral)

A. Nanoparticle vaccine design

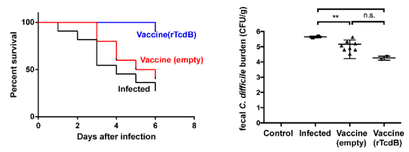
B. Oral Vaccination regiment and infection protocol



C. Induction of antigen-specific antibodies

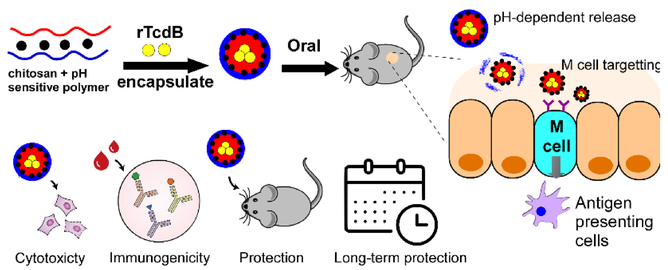


D. Immunization protected mice from *C. difficile* infection

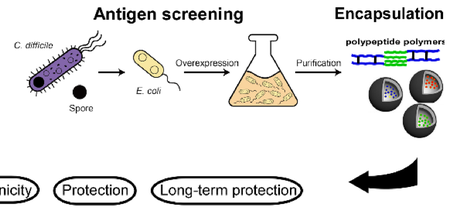


- Oral immunization was also successful in inducing robust antibody responses
- Immunization protected mice from infection
- Immunized mice still harbored higher level of C. difficile, thus immunized individuals might become asymptomatic carriers.

Next generation oral vaccine design:

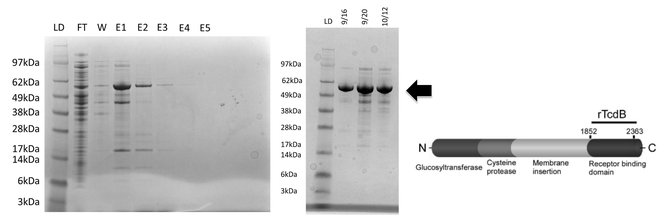


Name	Function
PilA	Type IV pili
SlpA	S-layer protein
FliC	Flagella
Cwp2	Cell wall protein
Cwp66	Cell wall adhesin
CbpA	Surface adhesin
Csp1	Surface adhesin
CdeC/M	Spore component



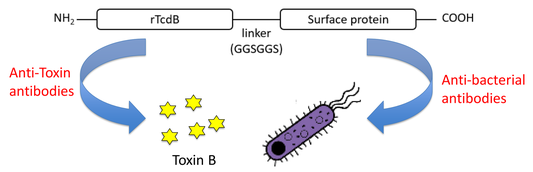
Focus 1: Evaluation of various *C. difficile* animal models

- Re-establishing animal infection models here at OSU-CHS (antibiotic cocktail vs single antibiotic method)
- Successful induction and purification of rTcdB as testing antigen (see below)
- Collaborating with Dr. Josh Ramsay (OSU) on Eudragit polymers



Focus 2: Chimeric protein as vaccine antigens

- *C. difficile* surface proteins as antigens
  - Type IV Pilin (tip protein) PilJ, S-layer protein SlpA
  - Flagellin protein FliC, Cell wall surface protein Cwp66
- Single vs chimeric protein design (See below)



Summary

- Pilot study demonstrated that IP delivered polymer vaccine induced specific humoral immunity
- Further work found oral vaccination with *rTcdB* provided significant protection against *C. difficile* infections
- Immunized mice were protected but still harbored *C. difficile* in the gut – potential for asymptomatic tranmission
- New design will utilize acid-stable and M-cell targeting polymers
- *C. difficile* surface proteins (PilJ, SlpA, FliC, and Cwp66) are hypothesized to increase vaccination efficacy by decreasing bacterial load

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- I would like to thank Dr. Muia's lab for support with protein purification and I specifically would like to thank Frida Miranda for teaching me to use FPLC

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