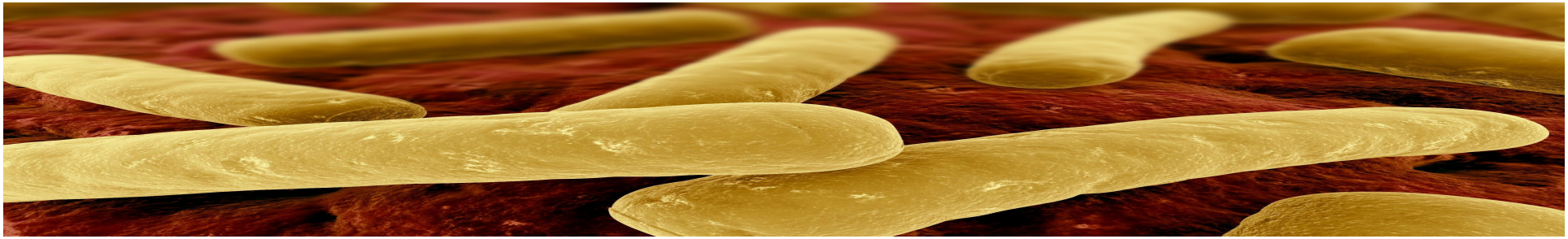

Novel chimeric vaccines against *Clostridium difficile* infection

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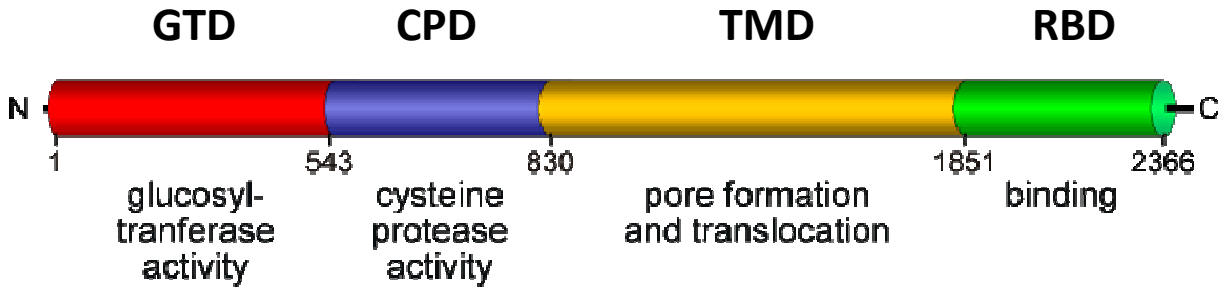
Clostridium difficile

- Gram positive, toxin-producing, spore-forming anaerobic bacterium.
- One of the three urgent antibiotic resistance threats in US.
- Leading cause of nosocomial antibiotic-associated diarrhea (AAD) in developed countries.
- The most common organism to cause healthcare-associated infections in US, leading to 14,000 deaths per year.
- A continual rise in the incidence of severe *C. difficile* infection (CDI) has been observed worldwide.
- Emergence of more virulent strains like NAP1/BI/027: increased toxin production and sporulation; altered antibiotic resistance pattern; secretion of additional toxin.

Major virulence factors

- Toxin A (TcdA) and Toxin B (TcdB): potent cytotoxic enzymes that damage the human colonic mucosa.



- Binary toxin (CDT): present ~6% of *C. difficile* isolates, but found in all hypervirulent strains.

Major colonization factors

- Two *C. difficile* flagellar proteins FliC and FliD are involved in the attachment of the organism to host cells and mucus layer.

- Serum antibody responses against both FliC and FliD are detected in patients with *C. difficile* infection.

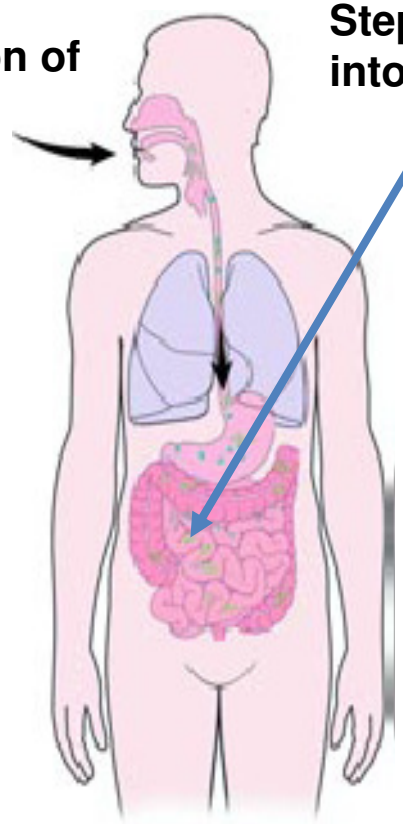
- *C. difficile* Cwp84 is a cysteine protease, and plays a critical role in the maturation of surface-layer proteins.

- Immunization with Cwp84 provides significant protection in hamsters by delaying *C. difficile* colonization. Cwp84 is highly immunogenic and conserved.

CDI: transmission through spores

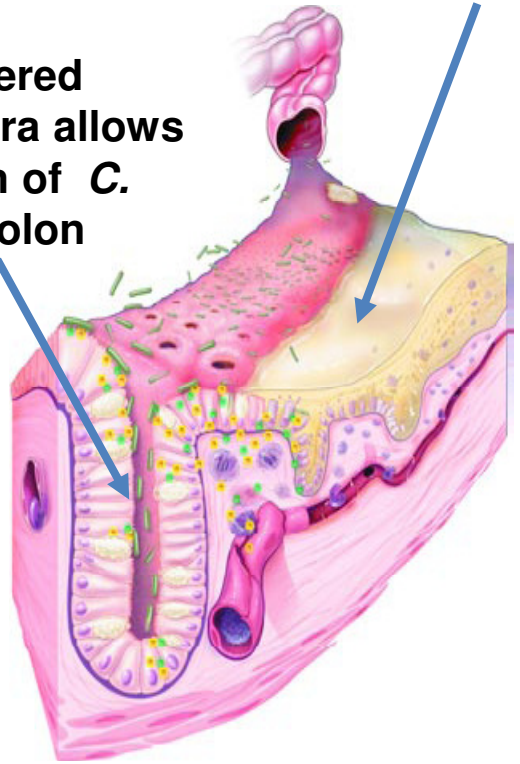
Step 1- Ingestion of spores

Step 2- Germination into vegetative cells



Step 3 - Altered intestine flora allows proliferation of *C. difficile* in colon

Step 4 . Toxin production leads to colon damage +/- pseudomembrane



CDI: Standard treatment and major challenges

Antibiotics: Standard treatment

- Vancomycin (only FDA-approved treatment)
- Metronidazole (most commonly used treatment)
- Fidaxomicin (lower recurrence rates)

Recurrence is common

- ~20% after first CDI episode
- ~40% after first recurrence
- ~60% after 2 or more recurrences

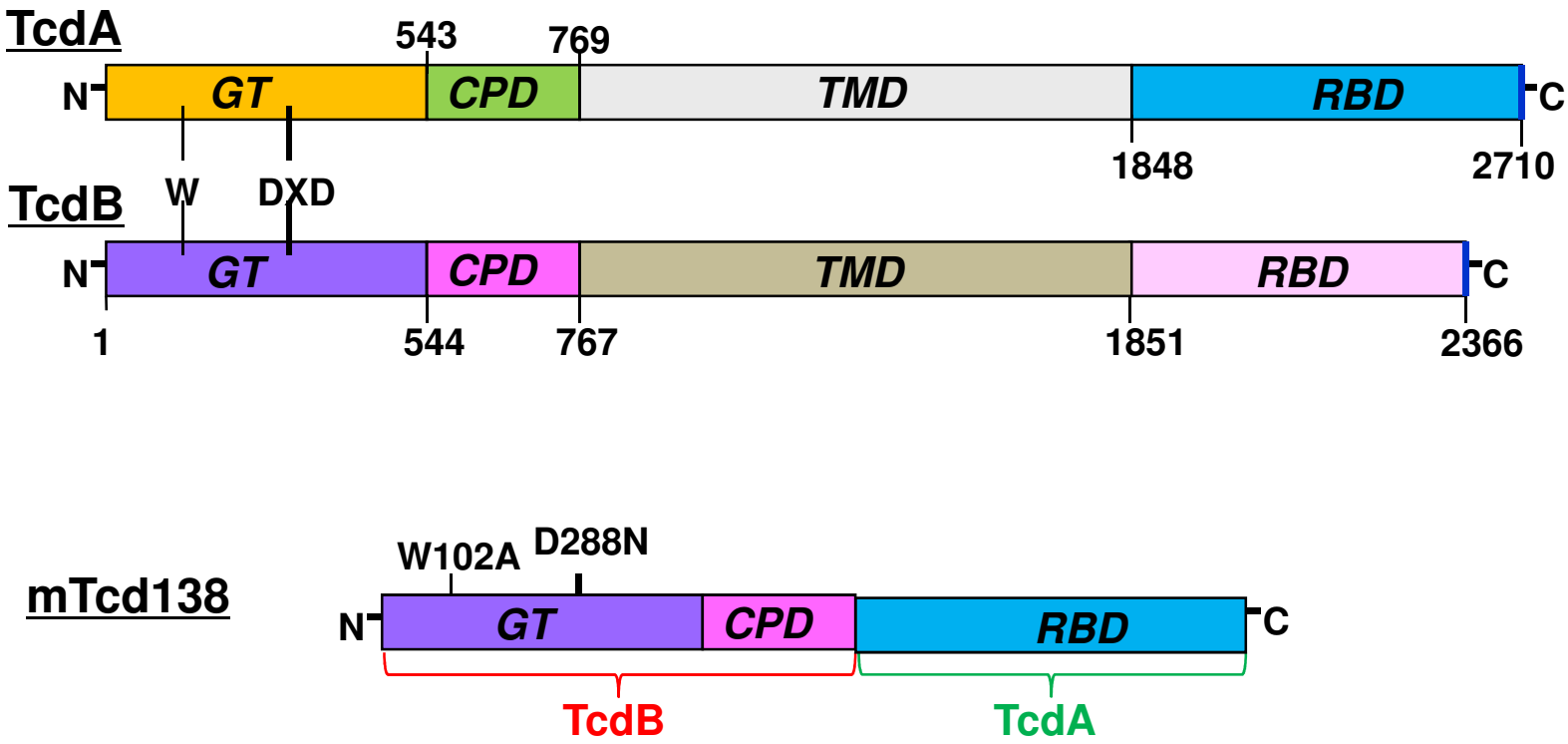
No vaccine against CDI is currently licensed

"*C. difficile* vaccination could be cost-effective over a wide range of *C. difficile* risk, especially when being used post-CDI treatment to prevent recurrent disease."

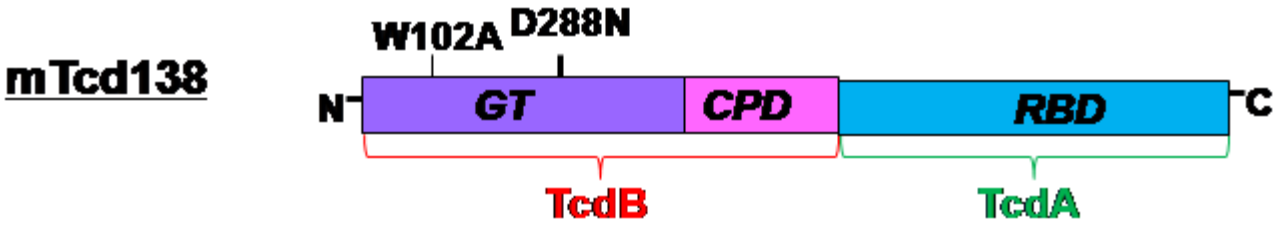
Lee et al., Vaccine, 2010 Jul 19;28(32):5245-53

The potential value of *Clostridium difficile* vaccine: An economic computer simulation model.

Novel vaccine candidate targeting both toxins: mTcd138

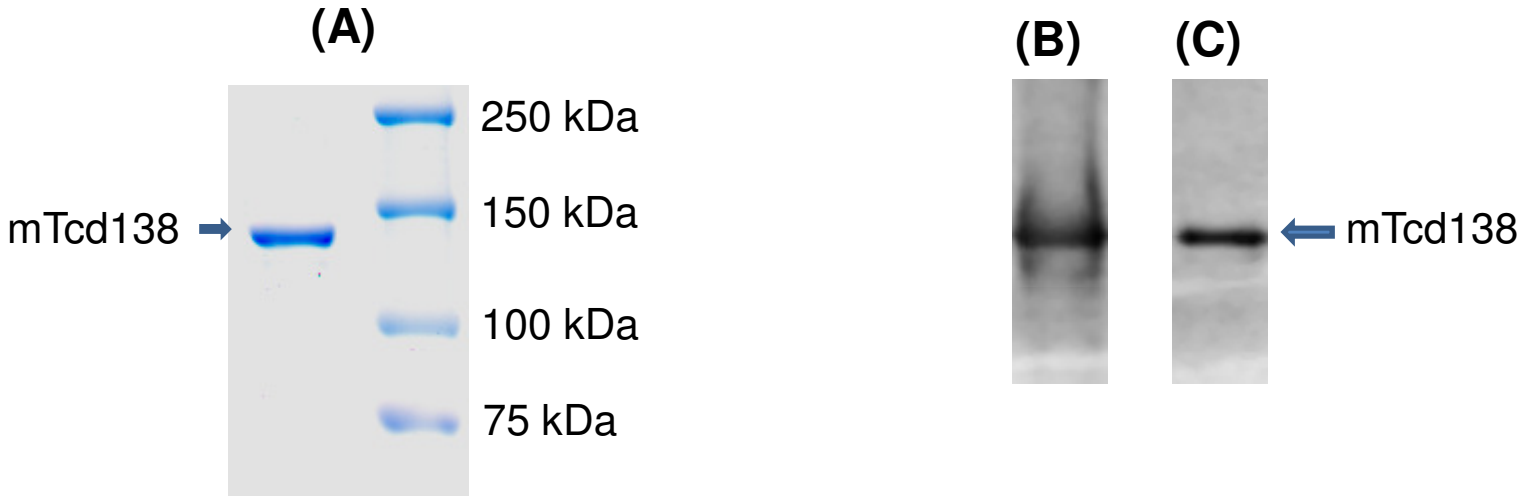


Construction of mTcd138: fusion of the N terminus of TcdB with the receptor binding domain (RBD) of TcdA



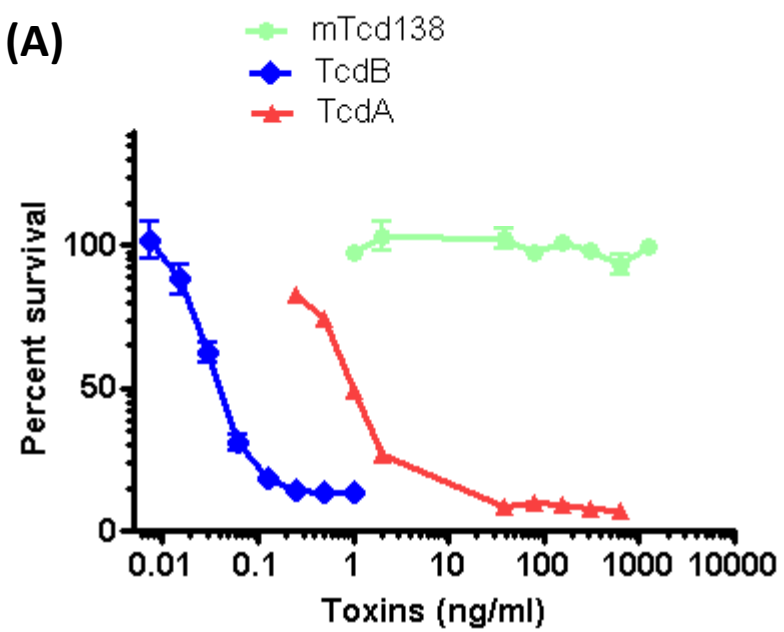
- The RBD is the immunodominant region of TcdA and has potent adjuvant activity.
- The neutralizing epitopes in TcdB are primarily confined to the N terminus since preincubation of polysera from the atoxic TcdB-immunized mice with recombinant TcdB-RBD did not significantly reduce the serum neutralizing activity.
- The N terminus in TcdB is more highly conserved between historical and hypervirulent strains than its RBD.
- Neutralizing antibodies to the TcdA-RBD were shown to be cross-reactive with TcdB-RBD.

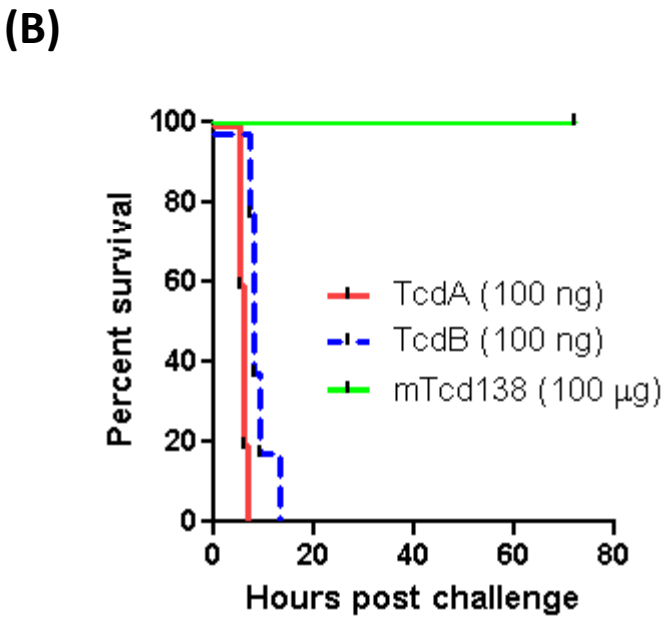
Expression and purification of mTcd138



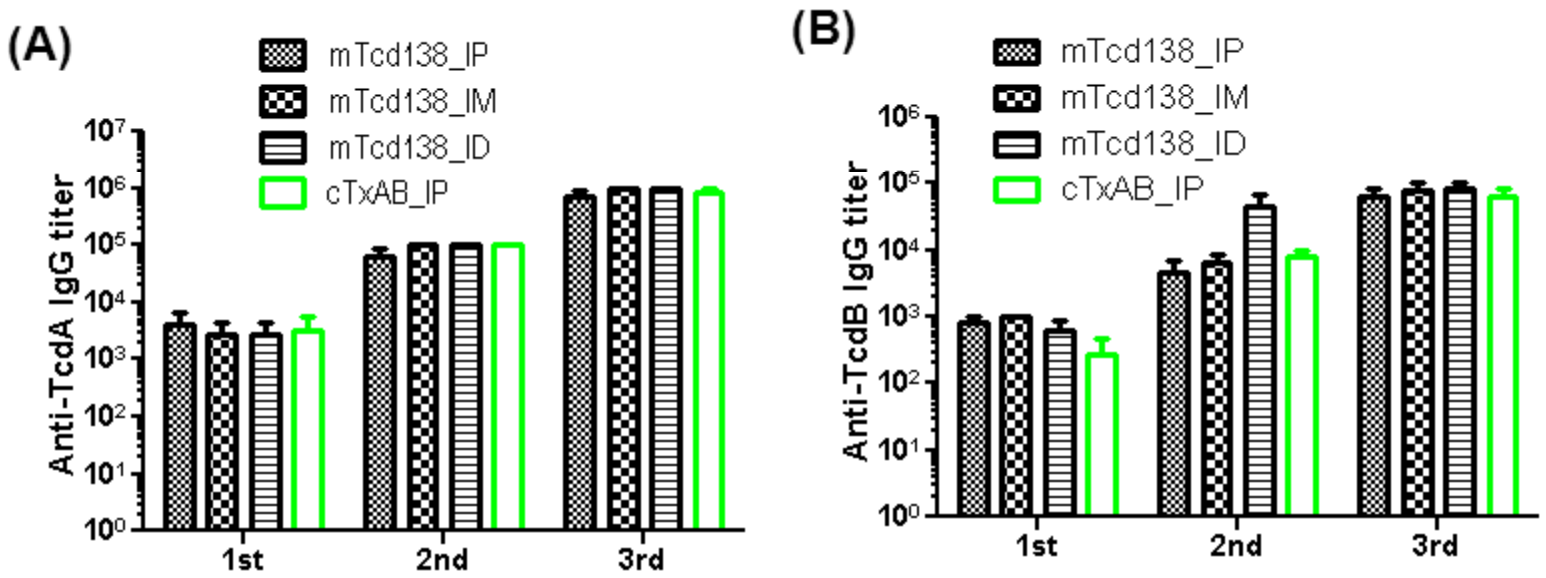
Analysis of purified 138 kDa fusion protein by SDS-PAGE **(A),** and Western blot analysis with anti-TcdA antibody **(B)** and anti-TcdB antibody **(C)**

mTcd138 is atoxic

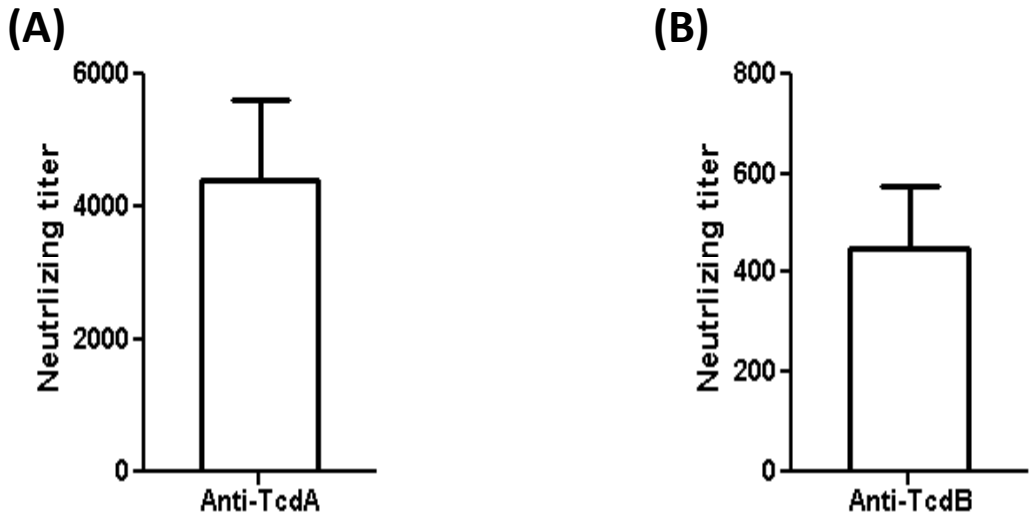




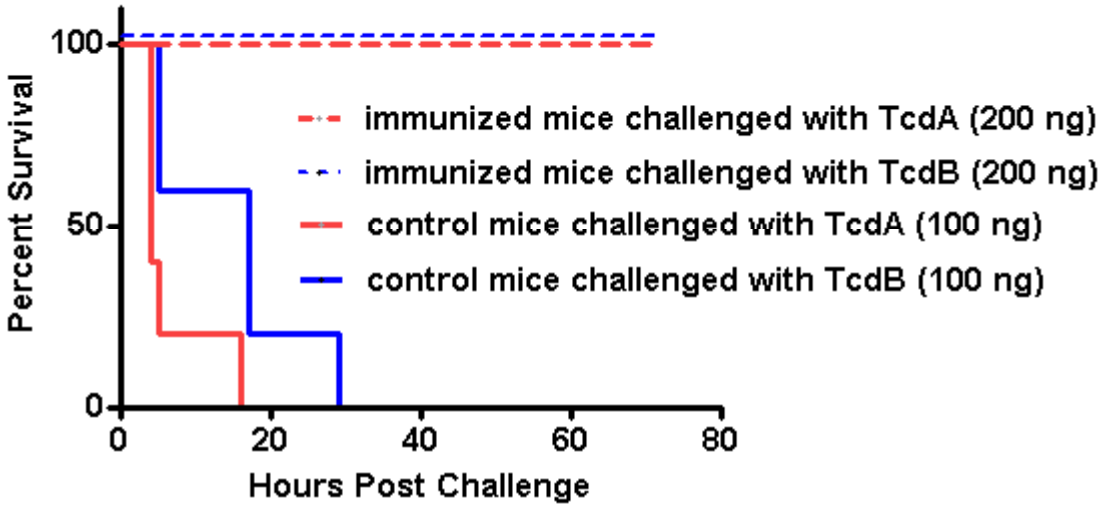
mTcd138 immunization via intraperitoneal (i.p.), intramuscular (i.m.) or intradermal (i.d.) routes induces similar levels of antibody response



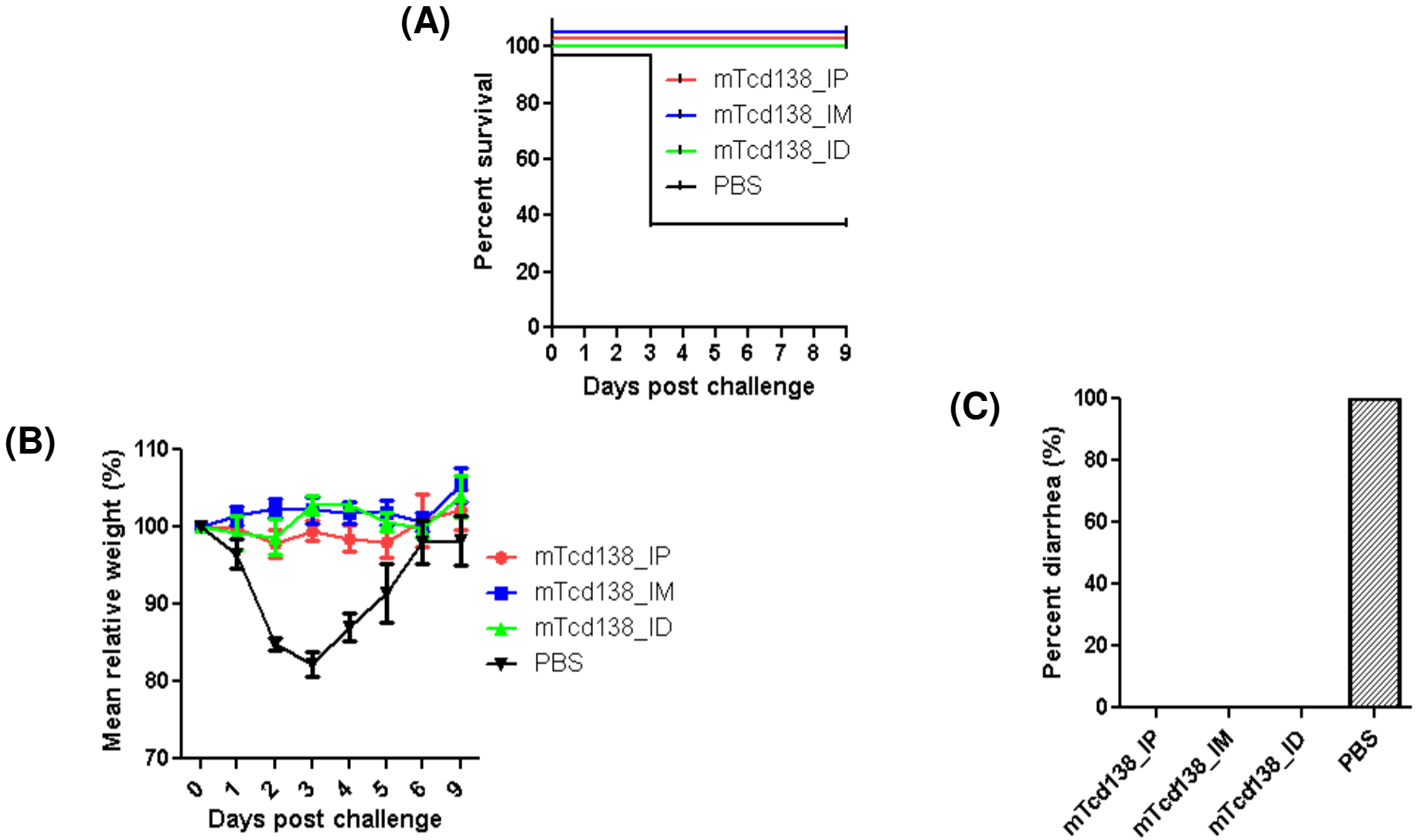
mTcd138 immunization induced potent neutralizing antibodies against both toxins



mTcd138 immunization protected mice against systemic toxin challenge



mTcd138 immunization of mice provided full protection against infection with a hypervirulent *C. difficile* strain

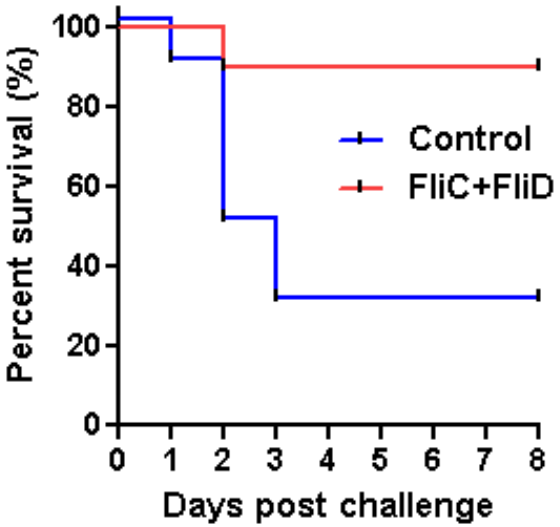


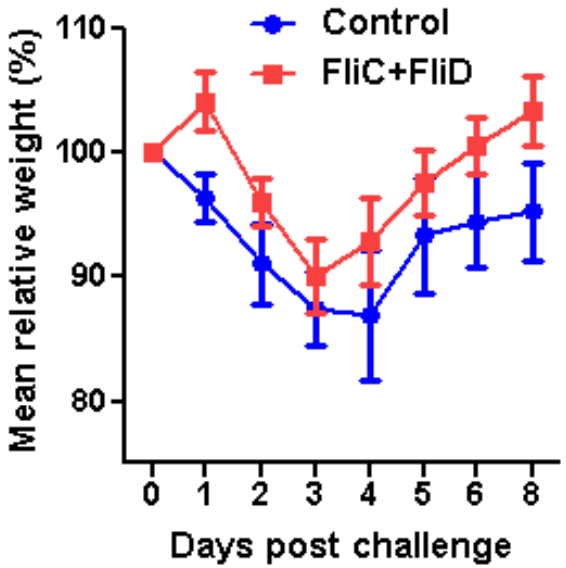
Development of a vaccine which targets *C. difficile* colonization and reduces *C. difficile* excretion

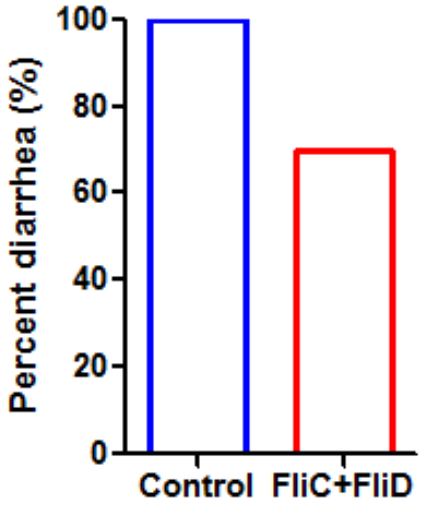
- Two *C. difficile* flagellar proteins FliC and FliD are involved in the attachment of the organism to host cells and mucus layer.

- Serum antibody responses against both FliC and FliD are detected in patients with *C. difficile* infection.

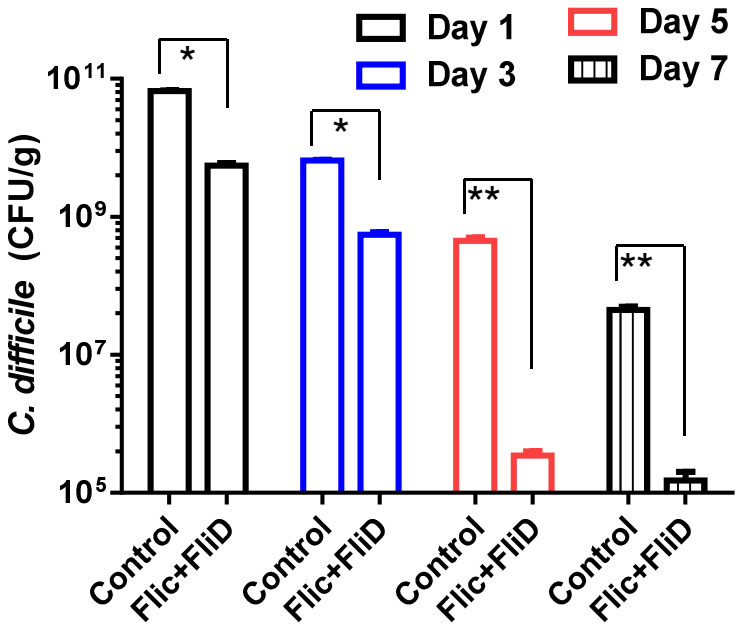
FliCFliD immunization of mice provides significant protection against infection with a hypervirulent *C. difficile* strain







FliCFliD immunization significantly reduces *C. difficile* excretion from mice



Summary of vaccine development

- We have constructed a novel vaccine targeting both *C. difficile* toxins.
- Immunization with mTcd138 induced potent antibody and protective responses against both TcdA and TcdB.
- We have generated a novel vaccine (FliCFliD) which can significantly reduce *C. difficile* colonization and excretion.

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