Highly immunogenic C-terminal binding domain of Clostridium difficile toxin a stimulates dendritic cell maturation

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Missions of Vaccine Center

1. To establish the infra-structure and facility for conducting vaccine research and development to meet regional needs,

2. To build and implement cGMP facilities in compliance with FDA regulatory and quality guidelines for manufacturing vaccines, vaccine candidates and anti-venom for regional use,

3. To develop the ability to respond to Taiwan government emergency requests for vaccines against pandemic diseases and bioterrorism, and

4. To serve as the forum for training and educating young scientists in vaccine-related biotechnology.

1. Taiwan Vaccine Self-Manufacturing program (2004-2007)
Roles of Vaccine Center

- **Up-Stream**
  - Academic
  - Identify Target

- **Mid-stream**
  - NHRI VRDC
  - Small Animals
  - Process Development
  - Primate or Permissive Animals
  - Clinical Phase I

- **Down-Stream**
  - Industry
  - Clinical Phase II
  - Clinical Phase III
  - Licensure

7-13年

圖 1 疫苗開發流程及相關機構
Vaccine Research and Development Center

Vaccine Center & cGMP facility

NHRI R1-7F (R&D)
**Clostridium difficile**

- A gram-positive bacteria
- A anaerobic, spore-forming rods (bacilli)
- Present as one of the 'normal' bacteria in the gut of healthy adults. It is much more common in babies (up to 70%).
- Eradication of the normal gut flora by antibiotics results with *C. difficile* colonization.

**C. difficile**-associated diarrhea (CDAD)  
Antibiotics-associated diarrhea are caused by **Toxin A** and/or **Toxin B**

**Scientific classification**

- **Kingdom:** Bacteria
- **Phylum:** Firmicutes
- **Class:** Clostridia
- **Order:** Clostridiales
- **Family:** Clostridiaceae
- **Genus:** Clostridium
- **Species:** C. difficile

**Binomial name**

*Clostridium difficile*  
Hall & O'Toole, 1935
Opportunistic infection in hospital

High recurrent risk
Double-edged sword of antibiotics in *C. difficile* treatment

Antibiotic treatment result two major concerns in *C. difficile* infection:

**Opportunistic infection after disruption of flora in small intestine**

**More virulent and antibiotics-resistant strain of *C. difficile* bacteria**

Source: Centers for Disease Control and Prevention, National Center for Health Statistics

Current antibiotics treatment:
metronidazole, vancomycin

Vaccine Development
Passive immunization:
Antibody-mediated antitoxin immunity
Sanofi in Phase 2 trials
Active immunization:
Vaccination to produce long-term protection
Sanofi used chemical inactivated whole toxin A as vaccine in phase 3
Subunit vaccine based on C-terminal repeat domain + adjuvant
Synthetic peptide based on the Repeating sequences + adjuvant
Recombinant chimeric toxin (A enzymatic domain + B RBD) + adjuvant
NHRI use lipoprotein based on the receptor binding domain of Cd Toxin
Aims of Vaccine Development: Preventing Diseases

Neutralize the toxins
Block the releases of toxins by tcde specific antibodies

(Modify from O'Connor JR et al., 2009)
Structure of *C. difficile* Toxin A and B

- **tcdD**: positive regulator
- **tcdB**: cytotoxin
- **tcdE**: putative holin function
- **tcdA**: enterotoxin
- **tcdC**: regulator

**Enzymatic Domain**: Glucosyltransferase activity, W102 & DXD

**Receptor-binding Domain**: Substrate Specificity, Putative Translocation Domain, CROPs
Lipo-TcdA-rRBD and TcdA-rRBD construction
rTcdA-RBD expression and purification

8% SDS-PAGE

LB medium, 20 °C induction, overnight
HA activity of Toxin A and rRBD in different concentration of rabbit RBC

Results had shown that rRBD had high HA activity than those obtained by Toxin A in rabbit RBC assay.
Assessing rRBD localization by confocal microscope
To evaluate **immunogenicity and biological function** of rRBD

Systemic antibody responses after BALB/c mice immunization with TcdA rRBD. (A) rRBD-specific IgG titer was continually monitored to 16th week by serum titer ELISA. (B) Isotype IgG were detected at 8th week mouse serum.
Mouse anti-RBD antibody recognized *C. difficile* toxin A
Mouse anti-rlipo-RBD sera could neutralize the toxicity of Toxin A in the Vero cell assay.
Neutralizing titer was defined as the reciprocal of the highest serum dilution that inhibited 100% cell rounding.

> 50% cell rounding


<table>
<thead>
<tr>
<th>Immunization</th>
<th>neutralization titer</th>
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<tr>
<td>PBS</td>
<td>&lt; 4</td>
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<tr>
<td>rRBD Lot 1</td>
<td></td>
</tr>
<tr>
<td>3 ug</td>
<td>8</td>
</tr>
<tr>
<td>10 ug</td>
<td>64</td>
</tr>
<tr>
<td>30 ug</td>
<td>64</td>
</tr>
<tr>
<td>rRBD Lot 2</td>
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<tr>
<td>30 ug</td>
<td>256</td>
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Protection against Toxin A toxicity in dose-response

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Percent survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>0%</td>
</tr>
<tr>
<td>0.3 ug rRBD</td>
<td>0%</td>
</tr>
<tr>
<td>3 ug rRBD</td>
<td>20%</td>
</tr>
<tr>
<td>30 ug rRBD lot 1</td>
<td>90%</td>
</tr>
<tr>
<td>30 ug rRBD lot 2</td>
<td>100%</td>
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</table>

Toxin A challenge dosage: 150 ng (5X LD50)
Design of a consensus sequence encoding tcdA rRBD and its fragments

A high homology and repetitive sequence: **35 short-term repetitive units**, (http://www.ebi.ac.uk/Tools/pfa/radar/)

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<table>
<thead>
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<th>No. of Repeats</th>
<th>Total Score</th>
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<th>Diagonal</th>
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<td>18</td>
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<td>302</td>
<td>319</td>
<td>1</td>
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</table>

**rRBD**

911 aa

104 kDa

f1 46.8 kDa
f2 43.8 kDa
f3 43.7 kDa
Purification of recombinant TcdA RBD and its fragments

(A) SDS-PAGE

(B) Western blot
  TcdA-specific Ab

(C) Western blot
  Anti-HIS tag
Mouse Immunogenicity of TcdA RBD Fragments

Functional antibody response against toxin A toxicity

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<tr>
<td>F1</td>
<td>8</td>
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<td>F3</td>
<td>16</td>
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<td>TcdA rRBD</td>
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TcdA RBD fragments could elicit functional antibody against TcdA toxicity.
Evaluation of dendritic cell maturation promoted by rRBD and activity of its fragments

<table>
<thead>
<tr>
<th>DC maturation markers</th>
<th>Cytokines</th>
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<tbody>
<tr>
<td>CD40</td>
<td>IL-6</td>
</tr>
<tr>
<td>CD80</td>
<td>IL-12</td>
</tr>
<tr>
<td>CD86</td>
<td>TNF-α</td>
</tr>
<tr>
<td>MHC-II</td>
<td></td>
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</tbody>
</table>
DC surface markers up-regulated by rRBD fragments

The mean fluorescence intensity (MFI) for cells culture in medium was defined as 100%
Cytokines secretion of DC stimulated by rRBD fragments
Evaluation of adjuvant effect of truncated RBD fragment

cdA rRBD fragments co-administration can enhance systemic OVA-specific IgG titer by intramuscular injection.
rRBD can
- agglutinate rabbit red blood cell (HA activity)
- bind and enter into the Vero cell
- alone elicit antibody responses that could inhibit TcdA toxicity
- at 30 ug dose elicit immune responses fully protecting mice from TcdA challenge
- up-regulate the immune effector molecules expression and stimulate cytokines release from dendritic cells
- function as an adjuvant (TLR-like agonist) to enhance anti-OVA antibody responses

All biological functions of rRBD are located at the C-terminal 320 amino acids
Mouse IgG elicited against rlipo-TcdA RBD or rRBD alone

Results had shown that rlipo-RBD alone was highly immunogenic even at one dose and 10X more potent than rRBD formulated in alum
**Clostridium difficile** Toxin A challenge

### Percent Survival

<table>
<thead>
<tr>
<th></th>
<th>0 hr</th>
<th>15 hr</th>
<th>24 hr</th>
<th>48 hr</th>
<th>Day 10</th>
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<tr>
<td><strong>PBS</strong></td>
<td>6/6 (100%)</td>
<td>0/6 (0%)</td>
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<td>0/6 (0%)</td>
</tr>
<tr>
<td><strong>30 ug TcdA-RBD</strong></td>
<td>10/10 (100%)</td>
<td>7/10 (70%)</td>
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<td>7/10 (70%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td><strong>30 ug rlipo-TcdA-RBD</strong></td>
<td>10/10 (100%)</td>
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謝 謝 Thank you!!
NSC grant: 101-2320-B-400-012
NSC grant: 101-2923-B-400-001