Toxin A and Toxin B Specific Systemic Antibody Levels Correlate with Protection against C. difficile Associated Disease in Hamsters

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**Clostridium difficile** Associated Disease

- *C. difficile* infection (CDI) is identified as the leading cause of nosocomial diarrhea and pseudomembranous colitis associated with antibiotic therapy¹.

- CDI incidence surpassed methicillin-resistant *Staphylococcus aureus* (MRSA) as the leading cause of hospital-acquired bacterial infection in the US and in Europe²,³.

- CDI is estimated to cause over $7 billion in healthcare costs annually in the US + EU⁴.

- Emergence and spread of a hyper-virulent strain BI/NAP1/027 (associated with increased morbidity and mortality⁵) since 2003 in North America, and later in Europe, is now becoming endemic⁶.

- Mortality rates from CDI have more than quadrupled in the US from 1999 to 2004⁷ and in the UK from 2004 to 2007⁶.

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¹Rupnik M, Wilcox MH, Gerding DN 2009; 7(7):526-36
²Kuijper EJ et al Clin Microbiol Infect 2006;6:2-18
³Miller et al Infect Control Hosp Epidemiol 2011;32(4):387-90
⁷Redelings MD et al Emerg Infect Dis 2007;13:1417-9
About *Clostridium difficile*

- A Gram-positive, spore-forming anaerobic bacillus
- Toxin A and B are major virulence factors for *C. difficile* disease
- Toxins A and B are similar
  - ~50% identity at amino acid level
  - High molecular weight (~300 kDa)
  - Domains
    - N-terminal enzymatic (Glucosyltransferase)
    - Central hydrophobic region
    - C-terminal binding domain (Receptor Binding)
- Serum anti-Toxin A and B IgG are associated with protection against recurrent CDI


Current treatment options and limitations

- **Current treatment options largely rely on antibiotics**\(^1\)
  - Metronidazole for mild to moderate CDI
  - Vancomycin for severe and recurrent CDI
  - Fidaxomicin recently approved and licensed (narrow-spectrum macrolytic antibiotic)\(^2,3\)

- **Limitations of current treatment options**
  - High recurrence rate\(^1,4\)
  - Increasing treatment failure\(^5\)
  - Selection of vancomycin-resistant enterococci\(^6\)

- There are no prevention products approved for use

\(^1\)Cohen SH et al *Infect Control Hosp Epidemiol* 2010;31:431-55; \(^2\)Optimer Pharmaceuticals (Dificid) [http://www.dificid.com/](http://www.dificid.com/)
Sanofi Pasteur’s approach to CDI

- Sanofi Pasteur’s *C. difficile* candidate vaccine is being developed for the prevention of primary disease caused by CDI\(^1\)

- The target population is adults at risk of CDI, such as those with planned hospitalization, long-term care/nursing home residents, those with co-morbidities that require frequent and/or prolonged antibiotic use\(^1\)

- Sanofi Pasteur’s *C. difficile* vaccine candidate contains highly purified, formalin-inactivated preparations of Toxins A and B
  - Alum-adsorbed, delivered via intramuscular injection
  - Has so far proven immunogenic and well-tolerated in adults and elderly
  - Currently is in Phase II clinical trials

\(^1\)Foglia G. et al *Vaccine* 2012;30:4307-9
Pre-clinical evaluation of Sanofi Pasteur’s \textit{C. difficile} vaccine candidate in hamster model

- Hamster is naturally susceptible to CDI and is a widely recognized model for \textit{C. difficile} associated disease

- Critical questions were addressed in hamster model
  - Immunogenicity and efficacy of the adjuvanted toxoid vaccine in active immunization/challenge studies
  - Correlation between binding and functional serum toxin-specific antibody responses and protection in active immunization/challenge studies
  - Efficacy of immune polyclonal sera against active components of \textit{C. difficile} vaccine in passive protection studies
Hamster efficacy model

- **Immunization (IM)**
  - Day 0, 14, 28
- **Clindamycin (IP)**
  - Day 34, 35
- **C. difficile (IG)**
  - Monitor weight loss, disease and survival

Indirect ELISA
- Toxin A
- Toxin B

IMR-90 Neutralization Assay
- Toxin A
- Toxin B

http://www.leinco.com/indirect_elisa

SJ. Demarest et al, mAbs 2:2, 1-9, 2010

Untreated
Immunization with the toxoid vaccine conferred protection against *C. difficile* challenge in a dose-dependent manner from challenge

- Animals immunized with decreasing doses of the vaccine were protected against death and disease in a dose-dependent manner from challenge
  - Animals challenged with *C. difficile* vegetative culture of the VPI10463 strain
  - Endpoint of the model >30% weight loss or morbidity
Immunization with the toxoid vaccine elicited serum Toxin A and B binding and neutralizing responses in a dose-dependent manner.

- Pre-challenge Toxin A and B antibody responses in the animal groups that received descending doses of the vaccine showed a dose-dependent pattern.
  - Pre-challenge antisera were tested in ELISA and IMR-90 neutralization assay using highly purified native C. difficile Toxins A and B.

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<tr>
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<th>Anti-Toxin A</th>
<th>Anti-Toxin B</th>
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<tr>
<td>ELISA</td>
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<tr>
<td>0.05 ug</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
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<td>0.5 ug</td>
<td>p&lt;0.0001</td>
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<td>5 ug</td>
<td>p=0.0009</td>
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<td>0.05 ug</td>
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<td>5 ug</td>
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ELISA toxin A and B specific serum IgG levels help to predict survival outcome in primary challenge model

- Median Toxin A- and B-specific IgG titers in the group of surviving animals were statistically higher compared to the group that died.
- The ELISA’s predictability of lack of protection was higher than predictability of protection.
- An ‘unprotective’ titer cutoff was established: 90% of animals with anti-toxin A titers ≤ 5.4 log10 EU/mL or anti-toxin B titers ≤ 5.3 log10 EU/mL succumbed to challenge.
Toxin A and B neutralizing titers in serum help to predict survival outcome in primary challenge model

- Median Toxin A- and B-neutralizing titers in the group of surviving animals were statistically higher compared to the group that died.
- Similar to ELISA, the neutralization assay’s predictability of lack of protection was higher than predictability of protection.
- An ‘unprotective’ titer cutoff was established: 90% of animals with anti-toxin A titers ≤3000 or anti-toxin B titers ≤240 succumbed to challenge.

**Graph:**
- Toxin A and B neutralizing titers (NT50) are plotted on a log scale.
- The x-axis represents the range of titers from 1 to 10,000.
- The y-axis represents the NT50 with dilution levels.
- The groups are divided into Protected and Unprotected.
- The data points are shown with box plots.
- The P-values are indicated: P < 0.0001∗.
Concordance observed between ELISA and Neutralizing titers for each component of vaccine

- Statistically significant correlation between the toxin-specific titers measured by ELISA and toxin neutralizing titers measured by IMR-90 cell-based assay was seen for both toxin A and toxin B responses by nonparametric Spearman test.
- Data confirmed that the toxin A and B binding serum antibodies correlated with functional (toxin neutralizing) activity.
Responses to both toxins were the best predictors of lack of protection in hamster efficacy model

With established titer cutoff, TcdA and TcdB ELISAs combined had high prediction value of lack of protection (True negative 87%) with low false positive rate (1%)

- False positive rates on the basis of either anti-TcdA (5%) or anti-TcdB (7%) only titers were slightly higher

- Assays had lower predictability of protection (True positive 46%) on the basis of both titers with higher false negative rate of 18%

- Assays had even lower predictability of protection on basis of either anti-TcdA or anti-TcdB only titer (33% and 3%, respectively)

- True positive rate on basis of anti-TcdA/B, anti-TcdA only and anti-TcdB only titers combined was 82%
Passive immunization with the anti-Toxoid A/B serum conferred protection against *C. difficile* challenge

Hamsters treated with one regimen of immune, but not normal, sera were protected against morbidity and mortality in a dose-dependent manner from *C. difficile* spore challenge (630* or VPI10463 strains)

- Immune serum pool was generated in hamsters by immunization with active components of the Sanofi Pasteur vaccine candidate
- Dose-dependent protection was observed for the challenge against both strains tested
Summary of findings

- Sanofi Pasteur’s *C. difficile* vaccine candidate delivered by intramuscular immunization elicited high Toxin A and B specific serum IgG response in hamsters

- The vaccine was efficacious in both active and passive protection models in hamsters
  - Critical role of toxin-specific circulating antibodies in protection was demonstrated

- Two *in vitro* readouts were identified as immune correlates of protection, toxin ELISA and IMR-90 neutralization assay
  - Demonstrated correlation between serum toxin-specific antibody levels and efficacy
  - Observed concordance between ELISA and neutralization assays for both Toxin A and B responses
  - Insufficient responses to both toxins were the best predictors of lack of protection suggesting importance of both components in vaccine
  - The assays had very low false positive and higher false negative rates
    - The false negative indications suggest that parameters other than toxin-specific Ab (such as duration of exposure, normal gut flora, response to non-toxin components of bacterium *etc* ) may play auxiliary role in protection
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