Clostridium difficile: pathogenesis, virulence factors and genetics

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Overview

• The major toxins, toxin A and toxin B.

• Binary toxin and 027 strains.

• The role of the major toxins in disease.

• Application of genetic tools to study virulence factors.

• The importance of spores for infection.
C. difficile pseudomembranous colitis

- Yellowish plaques of fibrin, mucus and inflammatory cells overlay the normal intestinal mucosa in pseudomembranous colitis.
- Gut damage is the result of toxin action.
**Clostridium difficile PaLoc**

- **tcdA** – Toxin A
- **tcdB** – Toxin B
- **tcdR** – positive toxin regulator
- **tcdC** – negative toxin regulator
- **tcdE** – involved in toxin release???

Large clostridial glucosylating toxins (LCTs)

- **PaLoc** (19.6 kb) is found in all toxigenic strains, absent in avirulent strains; important for strain typing.
Model of the uptake of C. difficile toxins

Toxins A and B:
Members of large clostridial glucosylating toxins family that glucosylate Rho GTPases.

Toxin action results in severe damage to intestinal epithelium.

Jank, T. et al., Glycobiology 2007 17:15R-22R
Genetic analysis to determine the role of toxins in disease

Kaplan-Meier survival curve of hamster trials with independently isolated toxin A and toxin B mutants – Toxin B is essential for virulence

Naturally occurring variant strains do not produce toxin A but do produce toxin B.

Hypervirulence and binary toxin (CDT)?

- 027 strains produce a third toxin, binary toxin (CDT), in addition to toxins A and B.

  - Found in approximately 10% of human isolates, but 23% - 100% of animal isolates.

- Correlation between CDT+ isolates and more severe disease; CDT is cytotoxic to Vero cells.

- Note: we now have mouse model of disease at Monash as well as 027 CDT mutants which will allow us to determine the role of this toxin in disease.
Hypervirulence and *C. difficile*

- Hypervirulent strains have recently emerged (NAP1/027).
  - More severe disease, increased mortality, wider patient demographic.
  - Epidemics in Canada, US, UK & EU
  - Produce CDT binary toxin
  - Resistant to fluoroquinolones
  - Produce 20-fold more toxin A/B
    - Possibly caused by mutation in *tcdC*
Hypervirulence and TcdC?

• There is an assumption that the “hypervirulence” of NAP1/027 strains is caused by the mutation in \( tcdC \).

• To conclusively determine the role of TcdC in NAP1/027 virulence ISOGENIC strains must be studied.

• Genetic analysis was used to determine the role of TcdC in the hypervirulent disease phenotype.
TcdC and NAP1/027 toxin production

- Provision of intact TcdC significantly represses toxin B expression 16 - 32-fold *in vitro*.

Vero cell cytotoxicity assay

- Chart showing toxin titre for different strains:
  - CD37
  - M7404 (WT)
  - DLL3001 (VC)
  - DLL3002 (tcdC')
  - DLL3003 (cured)

Toxin Titre (units)

Strain
The provision of \textit{tcdC} significantly reduces the virulence of NAP1/027 isolates. Suggesting that the \textit{tcdC} mutation is a contributory factor to the “hypervirulence” of these strains (unpublished data).
027 Epidemic Strains

- Multiple factors likely contribute to heightened virulence and not just the mutation in tcdC:
  - Variant toxins
  - CDT binary toxin
  - Antibiotic resistance
  - Enhanced sporulation and germination
  - Stronger, tougher, more persistent spores
C. difficile spores are the infectious particle
The human gastrointestinal tract and C. difficile

What is the sequence of events that lead to disease?

Nature. 2006 Dec 21;444:1009-10
Variant *C. difficile* strains will continue to emerge

The multidrug-resistant human pathogen *Clostridium difficile* has a highly mobile, mosaic genome


- Microarray and genome sequencing results showed that <20% of the genes in strain 630 are conserved among strains.
- These genes represent the core gene set.
- Results confirm the plasticity of the *C. difficile* genome, suggesting that variants will continue to emerge.
- Monitoring the strain variants in our hospitals is therefore critical for infection control, as has been found overseas.
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