ISOCENIC BINARY TOXIN C. difficile MUTANT SHOWS DECREASED ADHERENCE IN VITRO COMPARED TO THE PARENT (CDT+) STRAIN

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Abstract

CDT (Clostridium difficile toxinases) is a binary, actin ADP-ribosylating toxin frequently associated with hypervirulent C. difficile strains. This toxin is encoded by the CDT locus (CdtLoc), composed of cdtA, cdtB and cdtD. CDTs is the enzymatically active component, whereas the binding component. Together, CDTa and CDTb lead to the disassembly of the actin cytoskeleton, and later to cell death. Adherence to intestinal epithelial cells is the most probable initial step in the pathogenesis of C. difficile infection (CDT). It has been proposed that binary toxin CDT may enhance bacterial adherence and colonization by inducing the formation of microtubule-based protrusions on the surface of epithelial cells exposed to CDT.

To determine the effect of CDT on C. difficile adherence in vitro, we first constructed a cdtA-null mutant of the toxin A+-toxin B+CDT+ C. difficile strain, BK12 using Clostron technology. Functional ADP ribosylation activity was measured by quantifying incorporation of biotinylated NAD+ into HeLa cell lysate with exogenous actin, and was found to be completely abrogated in the mutant. Expression of the binding component of binary toxin (CDTb) was measured in 48h cultures by ELISA and was reduced 2.5-fold in the mutant compared to the wild type. Some expression of CDTb remained likely due to the intact cdtB gene in the mutant. We then compared in vitro adherence using the colonic epithelial cell line, SKCO-15. After 2h in an anaerobic chamber, planktonic C. difficile were removed and chum of adherent bacteria was calculated by spreading serial dilutions on TFA plates and counting resulting colonies. Percent adherence was determined for the BK12 wild type (CDT+) and BK12 mutant (CDT-) strains and with other well-characterized C strains. BI 17 and non-toxigenic M3 for comparison. Adherence varied greatly between strains with different genotypic backgrounds. However, the CDT-. BK12 mutant exhibited significantly decreased adherence (12%) compared to the wild type CDT+. BK12 strain (28%).

Disruption of CDT and abrogation of ADR ribosylation activity results in decreased epithelial cell adherence in vitro, and provides additional support for CDT as a virulence factor for C. difficile.

Assays and Results

Construction and confirmation of the binary toxin (cdtA) mutants: The parent strain, BK12, is a toxin A+, B+, CDT- strain that is typically typed by PCR ribotyping as 078/126 and associated with human and animal infections. The cdtA gene was interrupted using Clostron mutagenesis. The location of the primers chosen for confirmation of the mutants (M1-M6) and the PCR amplification are shown in the figure below.

Expression of the CDT binding component in the cdtA mutants: In vitro binary toxin production was measured in supernatants from BHI cultures incubated for different lengths of time. Culture supernatants were removed and cultured on TCF plates. Percentage adherence was calculated as: counts of bacteria recovered/bacteria added X 100.

Biologic activity of the cdtA mutants: Biologic activity of the mutants was measured using in vitro ADP ribosylation assays on the strain supernatants using HeLa cell protein and biotinylated NAD+. Following SDS-PAGE and Western blot transfer, ribosylated actin was detected using a streptavidin-HRP conjugate. The BK12 parent strain and control CDT-positive strains BI17 and BK12 strains (and AA1p to a lesser extent) demonstrated ribosylase activity. Three of the four BK12 binary toxin mutants (KO1,2 and 3b) expressed no detectable ribosylation activity.

Conclusions

- A mutant of the BK12 C. difficile strain was constructed in which the enzymatic component of the binary toxin gene (cdtA) was interrupted.
- The BK12 cdtA mutant strain did not ribosylate ADP.
- Residual production of the binding component of binary toxin, CDTb, was still present in the BK12 cdtA mutant.
- Adherence of the BK12 cdtA mutant to epithelial cell line was less than half of the adherence demonstrated for the BK12, CDT-positive parent strain.

Summary

Disruption of CDTa and abrogation of ADP ribosylation activity results in decreased epithelial cell adherence in vitro, and provides additional support for CDT as a virulence factor for C. difficile.

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