Microreview

In vivo virulence properties of bacterial cytolethal-distending toxin

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Summary

Multiple pathogenic Gram-negative bacteria produce cytolethal-distending toxins (CDTs). CDT is typically composed of three subunits: the catalytic subunit CdtB has DNase I-like activity, whereas CdtA and CdtC are binding proteins for delivering CdtB into target cells. Translocation of CdtB to the nucleus induces genotoxic effects on host DNA, triggering DNA repair cascades that lead to cell cycle arrest and eventual cell death. Several lines of evidence indicate that this toxin contributes to the pathogenicity of CDT-producing bacteria in vivo. Helicobacter hepaticus and Campylobacter jejuni CDTs are essential for persistent infection of the gastrointestinal tract and increase the severity of mucosal inflammation or liver disease in susceptible mouse strains. Haemophilus ducreyi CDT may contribute to the pathogenesis of chancroid in rabbits. Recently, H. hepaticus CDT has been shown to play a crucial role in promoting the progression of infectious hepatitis to premalignant, dysplastic lesions via activation of a pro-inflammatory NF-kB pathway and increased proliferation of hepatocytes, providing the first evidence that CDT has carcinogenic potential in vivo. Thus, both in vitro and in vivo data indicate that CDT is a bacterial virulence factor.

Introduction

Microbial pathogens, during co-evolution with their hosts, have developed various strategies for invasion, survival and adaptation in response to the environmental signals of the hosts (Bhavsar et al., 2007). One of these strategies is to block or subvert normal cellular functions of the host in order to create a better environment to avoid host defences and facilitate bacterial replication (Mattoo et al., 2007). Some examples of bacterial proteins that accomplish this in the gastrointestinal tract include cytolethal-distending toxin (CDT), and Helicobacter pylori CagA and VacA. Upon entry into target cells, these proteins modulate cell cycle progression to either inhibit or promote cell proliferation.

Bacterial CDTs belong to the AB2-type of toxins and generally comprise three subunits CdtA, CdtB and CdtC. Multiple pathogenic Gram-negative bacteria, including Aggregatibacter (formerly Actinobacillus) actinomycetemcomitans, Campylobacter spp., Escherichia coli, Haemophilus ducreyi, Helicobacter species, Salmonella enterica serovar Typhi (S. Typhi) and Shigella species, have been found to produce CDT (Haghjoo and Galan, 2004; Thelestam and Frisan, 2004). This toxin causes cell cycle arrest and eventual cell death in some cultured mammalian cells (Smith and Bayles, 2006). CdtB, after being delivered into the target cells with the binding of CdtA and CdtC on the cellular surface, utilizes its DNase I-like activity to induce limited host DNA damage [such as double strands breaks (DSB)], leading to activation of the DNA repair responses (Lara-Tejero and Galan, 2002). Much of our understanding of biological functions and genotoxic mechanism is based on studies using cultured mammalian cells, which have been in the subject of several recent review articles (Thelestam and Frisan, 2004; Smith and Bayles, 2006).

The in vivo pathogenic roles of bacterial CDTs are less clear; however, experimental evidence linking CDT to the virulence of bacterial pathogens is emerging. In this review, we briefly summarize the current knowledge of biology and functions of CDT based on in vitro and cultured cell systems, and then examine in more detail in vivo involvement of CDT in bacterial pathogenesis. Throughout the article, we will designate the individual CDTs and subunits as proposed by Thelestam and Frisan (2004): a specific CDT is represented by the initials of the producing bacterium before CDT: for example, HhCDT for Helicobacter hepaticus CDT, and Hhcdt for the H. hepaticus cdt operon; HhCdtA, HhCdtB, HhCdtC stands for the three subunits of HhCDT, which are
encoded by HhcdtA, HhcdtB and HhcdtC. In addition, five types of known CDTs in *E. coli* will be designated as EcCDT-I, II, III, VI and V respectively.

**In vitro characterization of CDT – overview**

**CDT – gene organization and biological functions**

Gene organization, a holotoxin complex and mechanisms of cytopathic effects for CDT are outlined in Fig. 1. All genetic loci encoding known CDTs contain three linked genes *cdtA*, *cdtB* and *cdtC*, with the exception of *S. Typhi* (see below) (Smith and Bayles, 2006). The *cdt* gene clusters are generally located on the chromosome of CDT-producing bacteria. However, the five EcCDTs can be encoded by chromosomal genes (EcCDT-II), chromosomal genes flanked with bacteriophage P2 and lambda-like sequences (EcCDT-V), an inducible lambdoid prophage in enteropathogenic *E. coli* (EcCDT-I) or a pVir plasmid in cytotoxic necrotizing factor II-producing *E. coli* strains (EcCDT-III) (Smith and Bayles, 2006; Asakura et al., 2007a). These subunits of CDT appear to be constitutively synthesized, assembled into a CDT complex and translocated into the periplasm in bacterial cells (Hickey et al., 2000; Ueno et al., 2006). The CDT complex is then secreted into the extracellular medium, probably via CdtA that undergoes post-translational cleavage at its N-terminal signal sequence (Hickey et al., 2000; Ueno et al., 2006). It has been documented that a proper complex of CdtA, CdtB and CdtC and its binding to the surface of the target cell are required for maximal cytotoxic activity (Heywood et al., 2005). Two host receptors

![Fig. 1. Diagrammatic outline of genetic organization, toxin complex, translocation and mechanisms of cytopathic effects for CDTs. See the text for details.](image_url)
have been implied in mediating the binding of CDT to the cell surface: fucose-containing glycoproteins for EcCDT-II (McSweeney and Dreyfus, 2005) and glycosphingolipid GM3 for AaCDT to the human monocyte cells (Mise et al., 2005). In addition, cholesterol-rich cytoplasmic membrane lipid rafts are also involved in the delivery of CdtB into target cell (Boeszze-Battaglia et al., 2006). Whether these different binding mechanisms are cell type-specific, CDT-specific or simultaneously operable in a given cell remains to be further investigated.

Upon binding of CDT to the target cell surface where CdtA and CdtC likely remain associated with the membrane (Boeszze-Battaglia et al., 2006), CdtB is internalized into the cell, probably via receptor-mediated endocytosis, and translocated through Golgi apparatus and endoplasmic reticulum to eventually reach the nucleus (Heywood et al., 2005). The entry of CdtB into the target nucleus is mediated by a nuclear translocation signal domain(s) (Smith and Bayles, 2006). Nuclear CdtB induces DNA damage such as DSB that result in rapid ataxi-telangiectasia mutated (ATM) kinase autophosphorylation. This action leads to activation of Chk2 by phosphorylation. The activated Chk2-P phosphorylates CDC25C, which results in the loss of phosphatase activity of CDC25C. The CDC25C-P cannot dephosphorylate cyclin B/CDK1-P, a checkpoint for continuation of cell cycle from G2 to M phase. Thus, progression of the cell cycle is arrested at the G2/M phase. Alternatively, ATM activates p53, which then increases the production of the cyclin-dependent kinase inhibitor p21. p21 can inhibit activates p53, which then increases the production of the cyclin-dependent kinase inhibitor p21. p21 can inhibit growth of the target cell (Boesze-Battaglia et al., 2006). Whether this phosphatase activity represents binary functions of CDT and how hydrolysis of PI-3,4,5-P3 to P2 leads to G2/M arrest requires further investigations.

S. Typhi CdtB – a component of typhoid toxin

The genome of S. Typhi, a facultative intracellular pathogen, contains a cdtB gene but not cdtA or cdtC (Haghjo and Galan, 2004). Expression and CDT activity of the S. Typhi cdtB gene are induced only by internalization of the organism into host cells (Haghjo and Galan, 2004). S. Typhi CdtB forms a complex with S. Typhi PltA (persussis-like toxin A) and PltB (persussis-like toxin B), designated as ‘typhoid toxin’. PltA is an orthologue of the pertussis toxin ADP-ribosylating subunit ‘A’, whereas PltB shares amino acid sequence similarity to one of the five components of its heteropentameric ‘B’ (Spano et al., 2008). The typhoid toxin is packed into a transport carrier (a membrane structure called ‘puncta’), and transported out of the infected cells. CdtB causes intoxication and G2 arrest of the target cells by being delivered into the S. Typhi-infected cell (an autocrine pathway) or non-infected cells (a paracrine pathway) (Spano et al., 2008).

In vivo pathogenic roles of CDT

Our knowledge of the pathogenic roles of CDT in natural hosts or experimental animal models is relatively limited. Such information is crucial for elucidating pathogen-host interactions, dissecting a role of CDT in disease pathogenesis, and developing rational therapeutic strategies against CDT-producing bacterial infections. Recent studies of CDTs in several bacteria have suggested that CDT plays an important role in bacterial infection and virulence of microbially induced gastrointestinal diseases, hepatic tumorigenesis and aggravation of chancroid in animal models, providing new insight into the pathogenic roles of CDTs in vivo.

Pathogenic involvement of HhCDT in persistent infection, induction of intestinal inflammation and promotion of hepatocarcinogenesis

Helicobacter hepaticus is a murine pathogen that can induce chronic active hepatitis, hepatocellular carcinoma (HCC), typhlocolitis and lower bowel cancer in susceptible strains of inbred and genetically engineered mice (Fox et al., 1994; Rogers and Fox, 2004; Rao et al., 2006).
Genetically, *H. hepaticus* is closely related to human bacterial pathogens *H. pylori* and *Campylobacter jejuni* (Suerbaum et al., 2003). CDT consisting of CdtA, CdtB and CdtC was first identified in *H. hepaticus* in 2000 by Young et al. (2000a). In addition, the cdtB orthologue and CDT activity are found in several other enterohepatic helicobacters, including *Helicobacter bilis*, *H. canis* and *H. marmotae* as well as the human isolates, *Helicobacter cinaedi* and *H. pullorum* (Chien et al., 2000; Young et al., 2000b; Fox et al., 2002; Taylor et al., 2003). Like other CDTs (Smith and Bayles, 2006), overexpressed, purified HhCdtB exhibits a DNase I activity that is abolished by mutations in HhCdtB at the predicted active amino acid residues conserved among the DNase superfamily (Avenaud et al., 2004). All three subunits are required for cytotoxic activity in CCL-9.1 cells (Avenaud et al., 2004). Young et al. (2004) created a CDT-negative isogenic mutant (3B1::Tn20, a mutation in cdtB) and a CDT-producing isogenic mutant (3B1::Tn16, a mutation downstream of the cdt operon). Although the CDT-negative 3B1::Tn20 colonized the IL-10<sup>−/−</sup> C57BL/6 (IL10<sup>−/−</sup>) mice, the mice developed significantly less severe typhlocolitis compared with mice infected with CDT-producing wild-type 3B1 or 3B1::Tn16 by 6 weeks post inoculation (p.i.) (Young et al., 2004). In a subsequent study in IL10<sup>−/−</sup> mice, Pratt et al. showed that 3B1::Tn20 had delayed colonization dynamics compared with 3B1, with loss of infection by 75 days p.i., and no detectible organisms by 225 days p.i. as judged by PCR and nested PCR, whereas wild-type 3B1 were still present at 8 months p.i. (Pratt et al., 2006). In addition, 3B1::Tn20-infected mice developed minimal intestinal inflammation, baseline Th2-associated IgG1 response, and weaker Th1-associated IgG2c response to infection compared with the 3B1-infected mice (Pratt et al., 2006). Furthermore, the mice that cleared infection with 3B1::Tn20 were partially protected from subsequent re-challenge with 3B1 or the same mutant (5 of 10 rechallenged mice cleared infection by 35 days p.i.) (Pratt et al., 2006).

Our group generated an independent EZ::TnCm-mediated, cdtB-deficient isogenic mutant (HhcdtBm7) of *H. hepaticus* 3B1 (Ge et al., 2005). In outbred Swiss Webster (SW) mice, females infected with the CDT-negative mutant cleared the infection by 8 weeks p.i., whereas the infected male mice had colonization levels of caecal and colonic HhcdtBm7 comparable to those of wild-type *H. hepaticus* 3B1-colonizing male and female mice (Ge et al., 2005). However, by 16 weeks p.i., HhcdtBm7 was no longer detected in the male SW mice, while all SW mice of both genders were colonized by 3B1. Clearance of HhcdtBm7 was associated with upregulation of ileal IFN-γ mRNA levels in female SW mice and caecal IFN-γ mRNA levels in males, suggesting that CDT may suppress IFN-γ response to helicobacter infection by inhibiting T-cell function as demonstrated for AaCDT in vitro (Smith and Bayles, 2006). We then investigated whether HhCDT is involved in tumour promotion in the liver of male A/JCr mice, which have been extensively used for dissecting the molecular mechanisms of *H. hepaticus*-induced hepatocarcinogenesis (Fox et al., 1996; Rogers and Fox, 2004). In male A/JCr mice, caecal levels of HhcdtBm7 were lower than levels of wild-type 3B1 through 4 months p.i. and were cleared in the majority of mice by 10 months p.i. (Ge et al., 2007). Interestingly, hepatic colonization levels of HhcdtBm7 were comparable to those of 3B1 at both 4 and 10 months p.i. Infection with the wild-type 3B1 or the CDT-negative mutant induced similar severity of hepatitis; however, only 3B1 infection led to the development of dysplastic, premalignant lesions at 10 months p.i. (Ge et al., 2007). When compared with infection with the CDT-negative mutant by 4 months p.i., infection with CDT-producing 3B1 enhanced hepatic mRNA expression of pro-inflammatory TNF-α, IFN-γ and COX-2 but not IL-1β; wild-type 3B1 infection also increased proliferation of hepatocytes and was associated with the increased mRNA levels of growth mediators IL-6 and TGF-α and anti-apoptotic Bcl-2 and Bcl-X<sub>i</sub> in the inflamed livers (Ge et al., 2007). Our data also showed that the 3B1-infected male mice significantly upregulated hepatic mRNA levels of RelA (p65), p50, GADD45β and c-IAP1, which are key components of the classical NF-κB pathway, compared with the CDT mutant-infected mice at 4 months p.i. (Ge et al., 2007). These results are consistent with the previous finding that activation of the classical NF-κB pathway in the liver is required for the progression of liver damage to HCC in *Mdr2<sup>−/−</sup> ΔlxB<sup>opp</sup> mice, an inflammation-associated HCC mouse model (Pikarsky et al., 2004). Through 10 months p.i., mRNA levels of hepatic IL-6, Bcl-X<sub>i</sub> and Bcl-2 were continuously higher in the 3B1-infected than the CDT-infected male A/JCr mice (Ge et al., 2007).

The pathogenic role of CDTs in enterohepatic bacteria, based mainly on the data regarding HhCDT, is outlined in Fig. 2. The production of CDT is apparently essential for persistent colonization of intestine by *H. hepaticus*, probably by the avoidance of the host immune surveillance via its cytotoxic effects on antigen-presenting cells, T and B lymphocytes. In addition, CDT is involved in elevating pro-inflammatory Th1-type immune response to infection, which leads to intestinal or hepatic inflammation in susceptible mouse strains. In the microbially induced HCC model, the presence of CDT enhances expression of a subset of pro-inflammatory mediators such as TNF-α, IL-6 and COX-2. Increased TNF-α likely activates the classical NF-κB pathway, which leads to increase expression of downstream growth mediators IL-6 and TGF-α compared with the CDT-negative mutant. These pro-inflammatory
cytokines and growth mediators in combination with the CDT-mediated suppression of apoptosis could be beneficial to survival of initiated hepatocytes in the early phase of the infection. During the course of bacterial infection, the sustained overproduction of IL-6 and anti-apoptotic proteins Bcl-2 and Bcl-XL facilitates proliferation of the initiated hepatocytes, thereby promoting the development of pre-malignant dysplastic lesions that ultimately give rise to HCC (Fox et al., 1996; Rogers and Fox, 2004).

CjCDT is essential for persistent gastrointestinal colonization of immunocompetent mice and also is involved in the induction of inflammation in the stomach, and intestine of immune-dysregulated mice

Campylobacter species are one of the most common bacterial causes of diarrhoeal illness in the USA, of which C. jejuni accounts for > 99% of reported Campylobacter infections (Mead et al., 1999; Allos, 2001). CDT is highly prevalent in isolates of various Campylobacter species and cytopathic effect can be significantly variable or even completely absent in some isolates (Smith and Bayles, 2006; Asakura et al., 2007b). The genotoxic effect of CjCdtB has been demonstrated in cultured mammalian cells and in yeast (Lara-Tejero and Galan, 2000; Hassane et al., 2001; 2003). Orogastric administration of culture supernatants of CDT-producing but not CDT-negative C. jejuni isolates to suckling mice (2 days old, two per isolate) induced overt inflammation in stomach, small intestine and large intestine (Jain et al., 2008). Severity was greater in mice inoculated with the wild-type strain, suggesting that CDT plays a crucial role in gastrointestinal pathology in this model. It is worth noting that the pathogenicity of individual C. jejuni isolates could be also influenced by genomic variations and slip-strand mutations within homopolymeric tracts of other genes (Parkhill et al., 2000; Taboada et al., 2004).
To investigate a role of CDT in the pathogenesis of _C. jejuni_, Purdy _et al._ created CDT-negative (cdtB) mutants of _C. jejuni_ 81-176 by an insertional mutation in _CjcdtB_, which were then characterized in immunodeficient SCID-Beige mice (lacking mature T and B lymphocytes). Five mice were infected with _C. jejuni_ 81-176 or the cdtB-mutant (Purdy _et al._, 2000). At 2 h p.i., _C. jejuni_ 81-176 was readily detected in 8 of 15 samples (including spleen, liver or blood) compared with 4 of 15 samples that were positive for the cdtB mutant, suggesting that CDT plays a role in systematic _Campylobacter_ infection. However, there was no difference in intestinal colonization levels or clinical signs by 7 days p.i. between the cdtB mutant- and _C. jejuni_ 81-176-infected mice (Purdy _et al._, 2000). In C57BL/129 mice, Fox _et al._ (2004) showed that the colonization ability of the cdtB mutant (four of eight) was comparable to its parental strain 81-176 (two of seven) at 2 months p.i.; however, the cdtB mutant was cleared in all of mice by 4 months p.i., while _C. jejuni_ 81-176 colonized 50% of the mice (four of eight). In contrast, in NF-κB-deficient mice (C57BL/129 background, p50+/−p65−/+ referred as 3X), colonization efficiencies between the cdtB mutant and 81-176 were similar at 2 and 4 months p.i.; however, the cdtB mutant did not induce gastroenteritis comparable to that developed in the _C. jejuni_ 81-176-infected mice at 4 months p.i. (Fox _et al._, 2004). Compared with C57BL/129, 3X mice colonized with both strains had significantly less total IgG and Th2-associated IgG1 humoral responses to _C. jejuni_ antigens. These results suggest that CDT may be required for _C. jejuni_ to escape immune surveillance of the immunocompetent C57BL/129 but is dispensable for persistent colonization of the immunodeficient 3X mice. In fact, NF-κB represents a group of transcription factors regulating inflammation, lymphoid organ development, B cell maturation and adaptive immunity (Bonizzi and Karin, 2004). Defects of these factors impair adaptive immunity involving mitogen and antigen-specific activation and function of mature B cells (Pohl _et al._, 2002).

In _Muc1+−/29/SvJ_ mice, McAuley _et al._ showed that the cdtB mutant, which was created by our group (Fox _et al._, 2004), had a significantly lower colonization level in the stomach but similar levels in the intestine, spleen and liver compared with _C. jejuni_ 81-176 at 2 days p.i. (McAuley _et al._, 2007). How CDT contributes to long-term, persistent colonization of these mice by _C. jejuni_ remains to be investigated. The possible role of CjCDT in pathogenesis has also been characterized in a chick model (Abououn _et al._, 2005). Infection of 1-day-old chicks with a CDT-negative mutant of _C. jejuni_ 81-176 did not influence colonization levels when compared with wild-type 81-176 and neither strain was able to cause disease. Intriguingly, despite the fact that the transcripts of cdt in the _C. jejuni_-infected chicks were detected by reverse-transcription PCR, no anti-CDT antibody response developed, whereas anti-CjCDT antibody was present in _C. jejuni_-infected human sera (Abououn _et al._, 2005). These results suggest that the chick model may not be suitable for investigating the role of CDT in _C. jejuni_ pathogenesis, because CjCDT appears to be more important in disease than in colonization, and unlike in mammals, _C. jejuni_ is a commensal in birds. Thus, animal models should be selected with these issues in mind when elucidating pathogenic mechanisms of CDT in other toxin-producing bacteria.

HdCDT implied pathogenicity in aggravating _H. ducreyi_-associated chancroid in rabbits

_Haemophilus ducreyi_, a fastidious Gram-negative bacterium, is the aetiological agent of the sexually transmitted disease chancroid. This disease is characterized by soft, painful and slowly healing genital ulceration; ~50% of chancroid cases are accompanied by inguinal lymphadenitis or bubo formation (Abeck _et al._, 1997; Cope _et al._, 1997). Chancroid is common in many developing countries and may facilitate HIV transmission (Lewis, 2003).

Cytolethal-distending toxin of _H. ducreyi_ is encoded by three linked chromosomal genes cdtA, cdtB and cdtC and in one study was produced by 82% of clinical isolates (Ahmed _et al._, 2001). An isogenic cdtC-inactivated mutant (cdtC mutant) of _H. ducreyi_ strain 35 000 lost the ability to produce CdtB but still maintained the ability to induce a lethal effect on HeLa cells and HaCaT keratinocytes (Stevens _et al._, 1999). Supernatants of the cdtB mutant of _H. ducreyi_ did not have a cytolethal effect on HeLa cells, whereas the cdtA mutant had moderate cytotoxicity on the same cell line (Lewis _et al._, 2001). Pathogenicity of these cdt mutants was characterized in a low-temperature rabbit model in which _H. ducreyi_ induces formation of necrotic skin lesions (Purcell _et al._, 1991). The _cdt_ genes were transcribed in the infected rabbits as demonstrated by reverse-transcription PCR (Lewis _et al._, 2001). Lack of expression of these individual CDT subunits did not affect the ability of the _cdt_ mutants to colonize the skin or to cause experimental lesions compared with its parental strain 35 000 at 2, 4 and 7 days p.i. (Stevens _et al._, 1999; Lewis _et al._, 2001). In addition, at infection sites of the upper arms of human volunteers, the pustule formation rates were similar for the _cdtC_ mutant and _H. ducreyi_ strain 35 000 (Young _et al._, 2001). These results suggest that CDT may not be required for chancroid lesion formation. However, intradermal inoculation with a CDT-negative _H. ducreyi_ strain in the low-temperature rabbit model, when co-administered with CDT, significantly increases the size and number of skin lesions compared with inoculation with _H. ducreyi_ alone, suggesting that this
toxin may contribute to the pathogenicity of _H. ducreyi_ at a high concentration (Wising _et al._, 2005).

**Concluding remarks and perspectives**

Epidemiological, _in vitro_ and _in vivo_ evidence indicates that CDT is a virulence factor that benefits bacterial survival and enhances microbial pathogenicity. Current experimental evidence supports the important role of CDTs in _in vivo_ pathogenesis of the CDT-producing bacteria, such as bacterial colonization and enterohemorrhagic bacteria-induced HCC and inflammation of stomach, intestine and liver in susceptible mouse strains during chronic infection. In contrast, the pathogenic role of CDT in animal models during the acute phase of infection has not been convincingly demonstrated so far. This may be at least in part due to the fact that the colonization dynamics and cytotoxic effects of CDT at a physiologically cellular level by some CDT-producing bacteria are not inflammation-associated during the acute infection interval and could become more apparent with chronicity. Whether the _in vitro_ findings of CDTs obtained in cultured cells are operable _in vivo_ needs to be examined. Precise mechanisms of how CDT affects bacterial pathogenicity, tissue inflammation and carcinogenesis induced by bacterial pathogens such as enterohemorrhagic bacteria warrant further elucidation. For example, CDT appears to be required for persistent colonization of murine intestine but not the liver by _H. hepaticus_ in male A/JCr mice. CDT-dependent persistent colonization did not induce intestinal inflammation, while CDT-producing but not CDT-negative _H. hepaticus_ induced dysplastic lesions in the inflamed liver (Ge _et al._, 2007). These results suggest that CDT could possess the ability to elicit multiple cytopathic effects in the host and the particular effect caused by CDT may indeed be niche-dependent. Therefore, it would be of interest in identifying environmental signals that regulate expression of _cdt_ transcriptionally and translationally, which will help to explain differential cytotoxic effects of a CDT. By infecting animal models with bacteria that produce chimeric or heterologous CDTs, one could ascertain how the different cytotoxic effects of the individual CDTs or subunits observed _in vitro_ contribute to _in vivo_ pathogenesis. Isogenic mutants of CDTs containing site-specific mutations can be used to define _in vivo_ biological functions of CDT at a molecular level, including modes of action, receptor specificity and cellular responses linked to the development of infectious diseases. Infections with various CDT mutants in combination with profiling the host responses by using molecular techniques such as microarray or with determining genes involved in inflammation and tumorigenesis by using RNAi, one could define the host signalling pathways or specific host factors which interacts with CDT. As CDT is essential for persistent colonization of the host, at least, by toxin-producing _H. hepaticus_ and _C. jejuni_ in mice, it could be of considerable interest to determine if anti-CDT vaccines generated with catalytic or receptor binding sites-mutated CDT subunits can prevent or clear infection of a human pathogen such as _H. ducreyi_. Some efforts have been made in this regard. For example, Lagergard _et al._ (2007) reported that formaldehyde treatment can increase the immunogenicity of HdCDT and decrease its toxicity. Taken together, further _in vitro_ and _in vivo_ investigations into CDT will not only increase the understanding of the virulence properties of CDTs but also could provide prophylactic and therapeutic strategies to combat infectious diseases such as diarrhea, inflammatory bowel disease, fever and chancroid.

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