Cytolethal distending toxin: a conserved bacterial genotoxin that blocks cell cycle progression, leading to apoptosis of a broad range of mammalian cell lineages

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Cytolethal distending toxin (CDT) is a heterotrimeric AB-type genotoxin produced by several clinically important Gram-negative mucocutaneous bacterial pathogens. Irrespective of the bacterial species of origin, CDT causes characteristic and irreversible cell cycle arrest and apoptosis in a broad range of cultured mammalian cell lineages. The active subunit CdtB has structural homology with the phosphodiesterase family of enzymes including mammalian DNase I, and alone is necessary and sufficient to account for cellular toxicity. Indeed, mammalian cells treated with CDT initiate a DNA damage response similar to that elicited by ionizing radiation-induced DNA double strand breaks resulting in cell cycle arrest and apoptosis. The mechanism of CDT-induced apoptosis remains incompletely understood, but appears to involve both p53-dependent and -independent pathways. While epithelial, endothelial and fibroblast cell lines respond to CDT by undergoing arrest of cell cycle progression resulting in nuclear and cytoplasmic distension that precedes apoptotic cell death, cells of haematopoietic origin display rapid apoptosis following a brief period of cell cycle arrest. In this review, the ecology of pathogens producing CDT, the molecular biology of bacterial CDT and the molecular mechanisms of CDT-induced cytotoxicity are critically appraised. Understanding the contribution of a broadly conserved bacterial genotoxin that blocks progression of the mammalian cell cycle, ultimately causing cell death, should assist with elucidating disease mechanisms for these important pathogens.

Introduction

Johnson and Lior’s seminal observations in the 1980s identified a novel heat-labile toxin in culture filtrates obtained from certain Escherichia coli, Shigella dysenteriae and Campylobacter jejuni strains which caused distinctive and progressive cytoplasmic and nuclear enlargement of cultured mammalian cells, so called cytolethal distending toxin (CDT), and uncovered a novel paradigm amongst bacterial toxins and virulence mechanisms (Johnson & Lior, 1987, 1988a, b). It was not until many years later that Scott & Kaper (1994) identified the genes encoding CDT in E. coli, which set the stage for fundamental investigations into the ecology, biochemistry and molecular mechanisms of cellular toxicity associated with this novel bacterial toxin (Table 1). While a secreted protein cytotoxin was identified among Haemophilus (Haem.) ducreyi clinical isolates in the early 1990s by Purvén & Lagergård (1992), it was not until the late 1990s that this cytotoxin was conclusively shown to be encoded by a cdt gene cluster with homology to the previously identified E. coli genes (Cope et al., 1997). This discovery extended the range of niches where CDT-producing bacteria are found to include mucocutaneous surfaces of the genital tract in addition to the intestinal tract. At that time, Péres et al. (1997) first reported that the mechanism of mammalian cell intoxication by E. coli CDT involved arrest of the cell cycle at the G2/M phase. Soon after, these observations were extended to CDT produced by Aggregatibacter (formerly Actinobacillus) actinomycetemcomitans (Sugai et al., 1998). Finally, Gelfanova et al. (1999) demonstrated that the underlying mechanism of CDT-induced cell death involved apoptosis.

The next milestone in CDT research came with the independent demonstration of structural conservation of amino acid residues and functional homology of CDTs from E. coli and C. jejuni to mammalian DNase I enzyme...
and correlation with DNA double strand breaks (DSBs) as the molecular basis of CDT-mediated mammalian cell cycle arrest (Elwell & Dreyfus, 2000; Lara-Tejero & Galán, 2000). With the understanding that CDT holotoxin consists of a heterotrimeric complex of CdtA, CdtB and CdtC subunits and reconstitution of the CDT holotoxin complex with individually expressed recombinant subunits, Lara-Tejero & Galán (2001) later proposed an AB toxin molecular model for CDT-induced cellular toxicity (as described below and by Lara-Tejero & Galán, 2001). This model has since been validated on the basis of high resolution crystal structure analysis of CDT from Haem. ducreyi and A. actinomycetemcomitans (Nesić et al., 2004). The identification of a cell membrane protein requirement for CDT binding to a myeloid leukemia cell line recently linked cell surface binding of the toxin to DNA damage (Carette et al., 2009). However, considering that CdtA and CdtC subunits have structural features consistent with lectin binding domains, the functional significance of a cell membrane protein as a component of the CDT receptor molecule remains to be clarified.

Currently, CDT is the only member of the bacterial AB toxins that exhibits DNase activity and, irrespective of the bacterial species of origin, exerts genotoxic damage by causing DSBs leading to irreversible cell cycle arrest and apoptosis in a broad range of mammalian cell lineages (Alouf, 2006; Dassanayake et al., 2005a; Frisan et al., 2003; Gelfanova et al., 1999; Hickey et al., 2005; Hontz et al., 2006a, b; Nesić et al., 2004; Scott & Kaper, 1994; Whitehouse et al., 1998; Yamada et al., 2006; Young et al., 2000b). The genes encoding CDT have now been found in more than two dozen bacterial species belonging to the Gamma- and Epsilon classes of Proteobacteria. Many of these Gram-negative bacteria are clinically important mucocutaneous pathogens of humans and animals that are responsible for major food- and water-borne bacterial illnesses worldwide (Table 2). It is expected that additional members of the classes Gamma- and Epsilonproteobacteria will be found to harbour CDT in the future.

The biological activity of CDT is highly dependent on cellular targets; however, haematopoietic cells are more susceptible, by several orders of magnitude, than all other cell types, suggesting a potential immunomodulatory role of CDT in the pathogenesis of diseases caused by CDT-producing bacterial pathogens. In this review, we highlight the ecology of pathogens producing CDT, outline the molecular biology of bacterial CDT, including current structural features and biological properties of the toxin, and critically address the molecular mechanisms of CDT-induced cellular toxicity. The respective roles of ATM and p53 in CDT-induced DNA damage response (DDR) leading to arrest of the cell cycle and ultimately cell death by apoptosis are reviewed in detail.

### CDT genes and encoded proteins

#### Distribution of CDT genes amongst bacteria

Currently, bacteria that harbour the CDT gene and display biological activity are restricted to the gamma and epsilon classes in the phylum Proteobacteria (Table 2). Within the Gamma proteobacteria, one subset belongs to the family Pasteurellaceae and includes Haem. ducreyi, the cause of a sexually transmitted disease known as chancroid (Abeck et al., 1997), Haemophilus parasuis, a commensal organism of the upper respiratory tract associated with septicaemia in pigs (Yue et al., 2009), and A. actinomycetemcomitans, a common contributing agent to chronic periodontitis and localized aggressive periodontitis (Henderson et al., 2002). A second subset of Gamma proteobacteria belongs to the Enterobacteriaceae family and collectively these organisms are responsible for intestinal and urinary tract infections that can lead to systemic spread. All members of the Epsilon proteobacteria belong to the order Campylobacterales and include several species of Campylobacter and enterohepatic Helicobacter species (EHS) which are primarily associated with enterocolitis; however, some species cause bacteremia/septicaemia, hepatitis and reproductive losses in humans and animals (Dassanayake et al., 2005a, b; Ge et al., 2008; Johnson & Lior, 1987; Liyanage et al., 2010). It is noteworthy that, in addition to mammalian bacterial pathogens, a monophyletic group of bacteriophage-encoded CdtB orthologues has been found amongst facultative endosymbionts of sap-feeding insects (Degnan & Moran, 2008). Thus, the ecology of CDT-harbouring bacteria is restricted to certain Gram-negative bacteria that primarily occupy mucocutaneous niches where persistent colonization can either occur as a commensal or result in localized or disseminated infections and diseases in a broad range of mammalian hosts.

<table>
<thead>
<tr>
<th>Discovery</th>
<th>Bacterium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological activity</td>
<td><em>Shigella dysenteriae</em></td>
<td>Johnson &amp; Lior (1987)</td>
</tr>
<tr>
<td>E. coli</td>
<td>Johnson &amp; Lior (1988a)</td>
<td></td>
</tr>
<tr>
<td>C. jejuni</td>
<td>Johnson &amp; Lior (1988b)</td>
<td></td>
</tr>
<tr>
<td>Haem. ducreyi</td>
<td>Purvén &amp; Lagergård (1992)</td>
<td></td>
</tr>
<tr>
<td>Gene sequence</td>
<td>E. coli</td>
<td>Scott &amp; Kaper (1994)</td>
</tr>
<tr>
<td>Cell cycle arrest</td>
<td>A. actinomycetemcomitans</td>
<td>Sugai et al. (1998)</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Haem. ducreyi</td>
<td>Gelfanova et al. (1999)</td>
</tr>
<tr>
<td>Nuclease activity</td>
<td>C. jejuni</td>
<td>Lara-Tejero &amp; Galán (2000)</td>
</tr>
<tr>
<td>Crystal structure</td>
<td>E. coli</td>
<td>Elwell &amp; Dreyfus (2000)</td>
</tr>
<tr>
<td>Cell surface receptor</td>
<td>Haem. ducreyi</td>
<td>Nesić et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>Carette et al. (2009)</td>
</tr>
</tbody>
</table>
Table 2. Gram-negative mucocutaneous bacteria harbouring CDT, their respective colonization niches and associated clinical diseases in human and animal hosts

EPEC, Enteropathogenic *E. coli*; ExPEC, extraintestinal pathogenic *E. coli*; APEC, avian pathogenic *E. coli*; UTI, urinary tract infection; NTEC, necrotoxigenic *E. coli*; EHEC, enterohaemorrhagic *E. coli*; STEC, shiga toxin-producing *E. coli*; NHP, non-human primates; HUS, haemolytic uraemic syndrome.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>CDT*</th>
<th>Host</th>
<th>Niche</th>
<th>Associated disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Class Gammaproteobacteria</strong></td>
<td></td>
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<td><strong>Family Pasteurellaceae</strong></td>
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</tr>
<tr>
<td><em>Haem. parasuis</em></td>
<td>HparCDT</td>
<td>Pig</td>
<td>Upper respiratory mucosa</td>
<td>Septicaemia</td>
<td>Yue <em>et al.</em> (2009)</td>
</tr>
<tr>
<td><strong>Family Enterobacteriaceae</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPEC/ExPEC</td>
<td>EcolCdtB-IV</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis/septicaemia</td>
<td>Tóth <em>et al.</em> (2003, 2009)</td>
</tr>
<tr>
<td>ExPEC</td>
<td>EcolCdtB-IV</td>
<td>Pig</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis/septicaemia</td>
<td>Tóth <em>et al.</em> (2003, 2009)</td>
</tr>
<tr>
<td><em>S. enterica</em> serotype Typhi</td>
<td>StypCdtB</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Typhoid fever</td>
<td>Haghjoo &amp; Galán (2004)</td>
</tr>
<tr>
<td><strong>Class Epsilonproteobacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td><strong>Family Campylobacteriaceae</strong></td>
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</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>CjejCDT</td>
<td>Cattle, sheep, pig</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Bang <em>et al.</em> (2003), Inglis <em>et al.</em> (2005)</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>CjejCDT</td>
<td>Sheep</td>
<td>Intestinal mucosa</td>
<td>Reproductive loss</td>
<td>Sahin <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>CjejCDT</td>
<td>Dog, cat, ferret</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Fox <em>et al.</em> (1987), Young &amp; Mansfield (2005)</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>CjejCDT</td>
<td>Chicken</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Young <em>et al.</em> (2007)</td>
</tr>
</tbody>
</table>
To simplify the designation of CDT produced by various bacteria, Cortes-Bratti et al. (2001a) proposed a nomenclature system which has been widely adopted by the scientific community. Since then, the list of bacteria that harbour CDT has expanded significantly (Table 2). Consequently, the original system consisting of the capitalized first letter of the genus followed by a single letter representing the species name has been modified to include the full species name in the nomenclature. This modification was introduced to avoid confusion and to provide a more comprehensive description of the bacteria involved in producing CDT.

Table 2. cont.

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>CDT*</th>
<th>Host</th>
<th>Niche</th>
<th>Associated disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. coli</em></td>
<td>CcolCDT</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Fouts et al. (2005), Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>CcolCDT</td>
<td>NHP</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Dassanayake et al. (2005b)</td>
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<td></td>
<td>CcolCDT</td>
<td>Cattle, pig</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Bang et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>CcolCDT</td>
<td>Sheep, chicken</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Garrity et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>CupsCDT</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis/bacteraemia</td>
<td>Fouts et al. (2005), Mooney et al. (2001), Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>CupsCDT</td>
<td>Pig, dog, cat, chicken</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Garrity et al. (2005)</td>
</tr>
<tr>
<td><em>C. upsaliensis</em></td>
<td>CupsCDT</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Edmonds et al. (1987)</td>
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<tr>
<td></td>
<td>CupsCDT</td>
<td>Pig, dog, cat, chicken</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Inglis et al. (2005)</td>
</tr>
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<td></td>
<td>CupsCDT</td>
<td>Cattle, pig</td>
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<td>CupsCDT</td>
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<tr>
<td><em>C. hyointestinalis</em></td>
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<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Fouts et al. (2005), Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>ChyoCDT</td>
<td>Cattle</td>
<td>Intestinal mucosa</td>
<td>Commensal</td>
<td>Johnson &amp; Lior (1988b), Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>ChyoCDT</td>
<td>Pig</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Ohya et al. (1993)</td>
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<tr>
<td><em>C. lari</em></td>
<td>ClarCDT</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Fouts et al. (2005), Pickett et al. (2006)</td>
</tr>
<tr>
<td><em>C. fetus subsp. fetus</em></td>
<td>CfetCDT</td>
<td>Human</td>
<td>Intestinal mucosa</td>
<td>Enterocolitis/bacteraemia</td>
<td>Garrity et al. (2005), Johnson &amp; Lior (1988b), Pickett et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>CfetCDT</td>
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<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Ohya et al. (1993)</td>
</tr>
<tr>
<td><em>C. fetus subsp. venerealis</em></td>
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<td>Intestinal mucosa</td>
<td>Enterocolitis</td>
<td>Asakura et al. (2008), Moolhuijzen et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>CvenCDT</td>
<td>Cattle</td>
<td>Urogenital mucosa</td>
<td>Reproductive loss</td>
<td>Garrity et al. (2005), Moolhuijzen et al. (2009)</td>
</tr>
</tbody>
</table>

**Family Helicobacteriaceae**

*Enterohepatic Helicobacter species*

| Hel. hepaticus         | HhepCDT  | Laboratory mice | Intestinal mucosa | Enterocolitis/ hepatitis | Young et al. (2000b) |
| Hel. bilis             | HbiliCDT | Laboratory mice | Intestinal/Biliary mucosa | Typhlocolitis/ hepatitis | Fox et al. (2004b), Fox (2007) |
|                       | HbiliCDT | Dog            | Intestinal mucosa | Commensal              | Hämminen et al. (2005), Kostia et al. (2003) |
| Hel. mastomyrinus      | HmasCDT  | Laboratory mice | Intestinal mucosa | Proctitis              | Shen et al. (2005) |
|                       | HmasCDT  | Mastomys       | Liver             | Hepatitis               | Shen et al. (2005) |
|                       | HcinCDT  | Human          | Intestinal mucosa | Septicaemia             | Taylor et al. (2003) |
|                       | HcinCDT  | NHP            | Intestinal mucosa | Colitis/hepatitis       | Fernandez et al. (2002), Fox et al. (2001) |
| Hel. canis             | HcanCDT  | Human          | Intestinal mucosa | Typhlocolitis           | Shen et al. (2009) |
|                       | HcanCDT  | Dog            | Intestinal mucosa | Bacteraemia             | Leemann et al. (2006) |
|                       | HcanCDT  | Laboratory mice | Intestinal mucosa | Enterocolitis/ hepatitis | Fox et al. (1996) |
| Hel. pullorum          | HpulCDT  | Human          | Intestinal mucosa | Enteritis               | Ceelen et al. (2006), Young et al. (2000a) |
|                       | HpulCDT  | Chicken        | Intestinal mucosa | Enteritis/hepatitis     | Ceelen et al. (2006), Young et al. (2000a) |
| Hel. winghamensis      | HwinCDT  | Laboratory mice | Intestinal mucosa | Commensal              | Boutin et al. (2010) |
|                       | HwinCDT  | Human          | Intestinal mucosa | Enteritis               | Melito et al. (2001) |

*CDT designation according to a modification of the nomenclature proposed by Cortes-Bratti et al. (2001a). Only the genes encoding CDT have been described in *Haem. parasuis* and *C. fetus subsp. venerealis*; the biological activity of CDT has not been determined in these bacteria.

†Variants of CdtB identified amongst pathotypes of *E. coli* are designated EcoCdtB-I to -V.
lower case species letter initial is no longer adequate, and some bacterial species now have overlapping designations; for example, Hp for Haemophilus parasuis and Helicobacter pullorum or Hc for Helicobacter cinaedi and Helicobacter canis. Therefore, we propose a modification of the previous nomenclature system in which the first three letters of the bacterial species in lower cases are placed after the capitalized first letter of the bacterial genus, followed by ‘CDT’ (Table 2).

CDT operon

The CDT holotoxin consists of a heterotrimeric complex of three subunits designated CdtA, CdtB and CdtC with corresponding approximate molecular masses of 23–30, 28–29 and 19–21 kDa according to bacterial host species (Haghjoo & Galán, 2004; Hu & Stebbins, 2006). In nearly all bacteria, CdtA, CdtB and CdtC subunits are encoded by adjacent or slightly overlapping cdtA, cdtB and cdtC genes which together form a constitutively expressed operon on the chromosome (Fig. 1). E. coli is unique amongst CDT-producing bacteria in that at least five divergent variants of CdtB have been found within this single species so far, which also differentially segregate according to E. coli pathotypes (Table 2; Janka et al., 2003). Since it is presently unknown whether or not the genes encoding the CdtA and CdtC subunits display sequence divergence similar to their corresponding CdtB variants, and until this can be resolved, we propose to designate these toxins as EcolCdtB-I to -V. Similar to other CDT-producing bacteria, EcolCdtB-I, -II, -IV and -V are encoded by chromosomal gene loci (Asakura et al., 2007a; Tóth et al., 2009), whereas homologues of bacteriophages P2 and lambda are found in E. coli strain O157:H– 493/89 harbouring EcolCdtB-V (Janka et al., 2003). These gene arrangements are reminiscent of bacteriophage-encoded CdtB orthologues found amongst facultative endosymbionts of sap-feeding insects in which acquisition of cdtB has been attributed to horizontal gene transfer (HGT; Degnan & Moran, 2008). Indeed, carriage of the CDT operon by certain enteropathogenic E. coli with EcolCdtB-I on a lysogenic phage background mediates HGT among related species, and confers enhanced bacterial toxicity during phage induction (Asakura et al., 2007a; Johnson et al., 2007; Oswald et al., 1994; Péres et al., 1997; Tóth et al., 2009). Also unique to E. coli is the location of the operon encoding EcolCdtB-III which is found on a large conjugative plasmid called pVir (Johnson et al., 2010). Finally, limited evidence to suggest that the cdt gene cluster of A. actinomycetemcomitans might have been part of a genomic island has been proposed (Doungudomdacha et al., 2007).

A notable exception to the heterotrimeric CDT model is Salmonella enterica serotype Typhi (S. Typhi) in which the genes encoding CdtA and CdtC are missing (Haghjoo & Galán, 2004). In keeping with our proposed CDT nomenclature, and since only the CdtB subunit is present in S. Typhi, the toxin should be designated StypCdtB. The cdtB gene of S. Typhi is located in a region of the chromosome with features consistent with a pathogenicity islet acquired by HGT and delineated by insertion sequences and a transposase gene (Haghjoo & Galán, 2004). Although the significance of these various CDT gene arrangements is incompletely understood, it is likely that they represent mechanisms of HGT which together might play a role in expanding the range of pathogenic bacteria that can establish persistent colonization and potentially cause infection and disease.

Fig. 1. Organization of the CDT gene locus. (a) In nearly all CDT-producing bacteria, the CDT gene cluster consists of three adjacent or slightly overlapping genes cdtA, cdtB and cdtC, encoding the corresponding CdtA, CdtB and CdtC protein subunits and is located on the chromosome, except for EcolCdtB-III in which the CDT gene cluster is found on a large conjugal plasmid called pVir. (b) In S. Typhi, the genes encoding CdtA and CdtC are missing; instead the StypCdtB protein subunit is encoded by the cdtB gene located upstream of pltA and pltB genes which encode PltA and PltB, respectively; homologues of the pertussis toxin ADP-ribosylating ‘A’ subunit and one of the five components of its heteropentameric ‘B’ subunit, respectively. Together, these are located on a pathogenicity islet delineated by insertion sequences (IS).
CDT protein structure and function

On the basis of structural and functional characteristics of reconstituted recombinant heterotrimeric CDT subunits, Lara-Tejero & Gala´n (2001) proposed an AB toxin molecular model for CDT-induced cellular toxicity (Lara-Tejero & Gala´n, 2001). Accordingly, the catalytically active ‘A’ subunit is attributable to the CdtB subunit, whereas the CdtA and CdtC subunits, which together display a high degree of sequence diversity, are assembled as a heterodimeric binding ‘B’ subunit mediating target host cell membrane specificity and cellular uptake of CdtB effector protein (Hu et al., 2006; Hu & Stebbins, 2006; Neˇsi´c et al., 2004). In S. Typhi, which lacks cdtA and cdtC, the holotoxin is instead composed of a single ‘B’ subunit and two ‘A’ subunits. The cdtB gene which encodes StypCdtB is located on a pathogenicity islet upstream of pltA and pltB genes, respectively, encoding pertussis-like toxin A and B (PltA and PltB) which are homologues of the pertussis toxin ADP-ribosyltransferase ‘A’ subunit and one of the five components of its heteropentameric ‘B’ subunit, respectively (Song et al., 2010; Spanò et al., 2008). PltA and PltB form a heterotrimeric complex with StypCdtB, where StypCdtB and PltA act as the ‘A’ or active toxic subunit, while PltB mediates the binding specificity or ‘B’ subunit function essential for toxin delivery and cellular toxicity (Song et al., 2010; Spanò et al., 2008). The holotoxin which displays both nuclease and ADP-ribosyltransferase activities has been referred to as ‘typhoid toxin’ (Song et al., 2010; Spanò et al., 2008).

Consistent with the notion that CdtB is the active subunit, comparative in silico structural analysis of predicted cdtB-encoded amino acid sequences of human and animal bacterial pathogens revealed conserved endonuclease/exonuclease/phosphatase super family domain with approximately 25–40 % sequence identity with phosphodiesterase enzymes including mammalian DNase I (Elwell & Dreyfus, 2000; Lara-Tejero & Gala´n, 2000). Despite the limited overall sequence homology with DNase I, cdtB-encoded catalytic, DNA- and divalent cation-binding residues are highly conserved among the various CDT-producing bacterial pathogens, suggesting a critical role for nuclease activity in host–pathogen interaction (Fig. 2; Elwell & Dreyfus, 2000; Hu & Stebbins, 2006; Lara-Tejero & Gala´n, 2000; Neˇsi´c et al., 2004).

The catalytic residues corresponding to mammalian DNase I-N170 (HducCdtB-N201) are highly conserved among all bacterial CdtB (Neˇsi´c et al., 2004; Pickett & Lee, 2005). One additional DNA-binding DNase I-R117 (HducCdtB-R41) residue, which is present in all CdtBs, has also been confirmed to be present in both AactCdtB and HducCdtB by mutational analysis (Neˇsi´c et al., 2004; Shenker et al., 2007). Differences in DNA-binding residue conservation between bacterial CdtB and mammalian DNase I probably account for reduced in vitro nuclease activities found with AactCdtB, EcolCdtB-II and HhepCdtB (Dassanayake et al., 2005a; Elwell & Dreyfus, 2000; Mao & DiRienzo, 2002). Additionally, differences in nuclease and cytotoxic activities of AactCdtB from clinical isolates have been attributed to a single amino acid substitution at position H281 (a residue not known to be associated with catalysis, DNA binding or divalent cation binding; Nishikubo et al., 2006).

More recently, high resolution crystallographic analysis of reconstituted heterotrimeric HducCDT holotoxin from individually expressed recombinant subunits (2.0 Å resolution; Neˇsi´c et al., 2004) or complete AactCDT operon expressed as a holotoxin in E. coli (2.4 Å resolution; Yamada et al., 2006) and an EcolCdtB-II subunit (1.73 Å resolution; Hontz et al., 2006b) have provided further support to earlier observations and confirmed that CdtB closely resembles mammalian DNase I. Comparative protein modelling revealed a high degree of key structural features conservation, namely the canonical four-layered fold of the mammalian DNase I family, the deeply grooved aromatic patch and the active site of CdtB from diverse bacterial species including CjejCDT, HhepCDT, EcolCDT-I, EcolCDT-II, EcolCDT-III, AactCDT and HducCdtB (Hu et al., 2006). While the heterotrimeric holotoxin forms a ternary complex between CdtB subunit and ricin-like lectin domains within CdtA and CdtB (Neˇsi´c et al., 2004), the lectin domains form a deeply grooved, highly aromatic patch which is critical for cellular toxicity, presumably through binding to target cell surface biomolecules (Neˇsi´c et al., 2004). Interaction between the N-terminal 13 amino acids of CdtC with the active nuclease site of CdtB suggests an auto-inhibitory function. Accordingly, the CdtB nuclease would be active only after release from CdtC and target cell entry.

Although the residues required for nuclease activity of CdtB are highly conserved, the overall amino acid sequence of CdtB varies among bacteria. While StypCdtB together with HducCdtB and AactCdtB form a distinct cluster, the CdtB produced by EHS and Campylobacter species forms a separate cluster which is distinct from CdtB produced by members of the E. coli/Shigella group (Degnan & Moran, 2008). Interestingly, a similar clustering pattern is seen when the amino acid sequences of CdtA and CdtC are compared between different bacterial pathogens (Eshraghi et al., 2010). The high degree of nucleotide and amino acid sequence homologies of CDT produced by Haem. ducreyi and A. actinomycetemcomitans as well as those produced by EHS and Campylobacter species suggests differential infection niches that may correlate with bacterial adaptation to...
persistent colonization in specific hosts. Alternatively, the possibility that these structural differences are indicative of variable bacterial target cell binding specificities and intracellular nuclease subunit signalling pathways remains to be determined.

Production of CDT

The prevalence of the CDT gene and biological activity varies among clinical isolates within individual bacterial species known to harbour CDT-producing members (Table 3). Assessment of clinical E. coli isolates for determination of EcolCdtB gene prevalence prior to current descriptions of the existence of variants I–V probably underestimated the percentage of strains harbouring the cdt gene operon (Ansaruzzaman et al., 2000; Clark et al., 2002; Janka et al., 2003; Johnson & Stell, 2000; Okeke et al., 2000; Toth et al., 2003). While the prevalence of the CDT gene and activity among clinical isolates of CDT-producing species of the families Pasteurellaceae, Campylobacteriaceae and Helicobacteriaceae is generally greater than 85%, a consistent finding with members of the Enterobacteriaceae family has been less than 14% prevalence. Considering the specificity of PCR screening, the possibility that additional variants could account for these discrepancies cannot be ruled out completely.

In addition to varying distribution among clinical isolates, the biological activity within individual bacterial species is variously found either in culture supernatant (and thus is presumably actively secreted), associated with bacterial cells or both (Table 4). Moreover, the activity of CDT recovered from these different fractions can range from relatively high, intermediate, low to absent, and this may be relevant to disease pathogenesis for individual bacterial pathogens. Similar to other virulence factors, the possibility that these alterations in CDT production are attributable to high numbers of passages on laboratory media and artificial selection of strains that are less virulent cannot be ruled out completely.

The early studies of Johnson & Lior (1988a) assessed CjejCDT in culture supernatant; however, assessment of cell-associated CDT among clinical C. jejuni isolates revealed relatively high biological activities in most strains with few strains exhibiting comparatively lower levels of activity (Pickett et al., 1996). Strains of C. jejuni that harbour the cdt gene cluster but lack CDT biological activity have also been found (Abouen et al., 2005; Dassanayake et al., 2005b). Two types of mutations within the cdt gene operon that can account for a lack of CDT activity have been found in these strains; deletion across...
Table 3. Prevalence of CDT gene and activity amongst clinical isolates of different bacteria

<table>
<thead>
<tr>
<th>CDT</th>
<th>No. positive/no. tested (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HducCDT</td>
<td>6/10 (60)</td>
<td>Purveén &amp; Lagergård (1992)</td>
</tr>
<tr>
<td>AactCDT</td>
<td>43/50 (86)</td>
<td>Ahmed et al. (2001)</td>
</tr>
<tr>
<td>EcolCdtB†</td>
<td>17/430 (5)</td>
<td>Bielaszewska et al. (2004)</td>
</tr>
<tr>
<td>SbocCDT</td>
<td>43/50 (86)</td>
<td>Ahmed et al. (2001)</td>
</tr>
<tr>
<td>SdysCDT</td>
<td>20/20 (100)</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td>CjejCDT</td>
<td>10/11 (90)</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td>CcolCDT</td>
<td>12/12 (100)</td>
<td>Asakura et al. (2007b)</td>
</tr>
<tr>
<td>CupsCDT</td>
<td>5/5 (100)</td>
<td>Asakura et al. (2007b)</td>
</tr>
<tr>
<td>HcinCDT</td>
<td>11/11 (100)</td>
<td>Taylor et al. (2003)</td>
</tr>
</tbody>
</table>

*Tested for cdtA, cdtB and cdtC by PCR.
†Recent reports that tested for the presence of all currently known variants (I–V) of EcolCdtB were included.
‡Tested only for cdtB by PCR.
§Non-O157 STEC: three CdtB-III and 14 CdtB-V were tested.
||STEC: seven CdtB-III and seven CdtB-IV were tested.

Information concerning the molecular mechanism of bacterial synthesis and secretion of CDT is limited (Deng et al., 2001; Ueno et al., 2006). A difference of approximately 2 kDa between the observed and calculated molecular masses of EcolCdtB-II subunit has been attributed to cleavage of a putative N-terminal signal peptide sequence involved in secretion across the inner membrane by a general export pathway (Dreyfus, 2003). Consistent with these observations, Ueno et al. (2006) demonstrated the presence of a lipid-binding consensus motif (lipobox) and lipoprotein cleavage site in AactCdtA signal peptide. In addition to AactCdtA, putative lipobox motifs are also present in HducCdtA, CjejCdtA, HhepCdtA, EcolCdtA-I, -II and –III, and EcolCdtC-I, -II and –III (Ueno et al., 2006; R. N. Jinadasa & G. E. Duhamel, unpublished observations). While the periplasmic CDT holotoxin consists of the uncleaved AactCdtA, AactCdtB and AactCdtC subunits, the secreted holotoxin complex in culture supernatant has cleaved AactCdtA (Ueno et al., 2006). A similar post-translational cleavage of HducCdtA probably accounts for the two forms with approximate molecular masses of 23 and 17 kDa seen with recombinant HducCdtA (Frisk et al., 2001).
Cell-associated CjejCDT is found primarily in the periplasmic space of *C. jejuni*, whereas culture supernatant contains primarily CjejCDT tightly associated with outer membrane vesicles (Lindmark et al., 2009). In the context of intestinal infection, packaging and release of CDT into outer membrane vesicles may serve a protective function against enzymic digestion, thus allowing uptake of intact protein toxin by host absorptive enterocytes. Further studies on the mechanism of CDT production and release by bacterial pathogens should provide important insights for the design of therapeutic modalities aimed at inhibiting CDT production or neutralizing CDT activity during infection.

**Molecular mechanisms of cellular toxicity**

**Cellular entry of CdtB**

A causal relationship between the requirement for heterotrimeric CdtABC holotoxin and cellular toxicity is well established for AactCDT, CjejCDT, EcolCdtB-I and -II and HducCDT (Akitfusa et al., 2001; Lara-Tejero & Galán, 2001; Pickett et al., 1994, 1996; Purvén et al., 1997; Scott & Kaper, 1994; Shenker et al., 2005). In these pathogens, CdtA and CdtC subunits bind host cell membrane lipid raft microdomains before internalization of CdtB (Fig. 3; Boesze-Battaglia et al., 2006, 2009; Carette et al., 2009; Cortes-Bratti et al., 2000; Lara-Tejero & Galán, 2001; Nešić

### Table 4. Production of CDT by bacterial pathogens

<table>
<thead>
<tr>
<th>CDT</th>
<th>Bacterial location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcolCdtB-I</td>
<td>High</td>
<td>Tóth et al. (2003)</td>
</tr>
<tr>
<td>EcolCdtB-II</td>
<td>Present</td>
<td>Pickett et al. (1994)</td>
</tr>
<tr>
<td>EcolCdtB-IV</td>
<td>Absent</td>
<td>Tóth et al. (2003)</td>
</tr>
<tr>
<td>EcolCdtB-V</td>
<td>Present</td>
<td>Bielaszewska et al. (2005), Janka et al. (2003)</td>
</tr>
<tr>
<td>ShoyCDT</td>
<td>Present</td>
<td>Hyma et al. (2005), Johnson &amp; Lior (1987)</td>
</tr>
<tr>
<td>SdysCDT</td>
<td>Present</td>
<td>Johnson &amp; Lior (1987)</td>
</tr>
<tr>
<td>CjejCDT</td>
<td>High</td>
<td>Dassanayake et al. (2005b), Pickett et al. (1996)</td>
</tr>
<tr>
<td>CcolCDT</td>
<td>Absent</td>
<td>Asakura et al. (2007b), Dassanayake et al. (2005b), Pickett et al. (1996)</td>
</tr>
<tr>
<td>CupsCDT</td>
<td>Present</td>
<td>Mooney et al. (2001), Pickett et al. (1996)</td>
</tr>
<tr>
<td>ChyoCDT</td>
<td>ND</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td>ClarCDT</td>
<td>ND</td>
<td>Pickett et al. (1996)</td>
</tr>
<tr>
<td>CfetCDT</td>
<td>Present</td>
<td>Asakura et al. (2007b), Ohyata et al. (1993), Pickett et al. (1996)</td>
</tr>
<tr>
<td>EHS</td>
<td>ND</td>
<td>Chien et al. (2000), Hänninen et al. (2005), Kostia et al. (2003), Shen et al. (2005), Taylor et al. (2003)</td>
</tr>
</tbody>
</table>

*CDT activity in unconcentrated culture supernatant.
†CDT activity in bacterial lysate or enriched outer membrane preparation.
et al., 2004; Shenker et al., 2005). The integrity of lipid membrane raft microdomains is critical for binding of AactCDT to human T-cell leukaemia Jurkat cells, presumably involving binding of host cell membrane cholesterol by a putative AactCdtC subunit cholesterol recognition/interaction amino acid consensus domain (Boese-Battaglia et al., 2009). As predicted, pretreatment of cells with CdtA–CdtC complex protects against intoxication by CdtABC holotoxin, but not by CdtB alone, again demonstrating that the internalization of CdtB is critical for toxicity (Deng & Hansen, 2003).

Structural analysis of CdtA and CdtC has revealed the presence of ricin-like lectin domains, suggesting that an interaction with host cell membrane carbohydrate molecules might mediate CDT binding (Eshraghi et al., 2010; Hu et al., 2006; Nešić et al., 2004). This is consistent with observations by McSweeney & Dreyfus (2005) demonstrating a critical role for cell surface N-linked glycoprotein playing a critical role in binding of EcolCdtA and EcolCdtC to cultured human epithelioid cervical carcinoma HeLa cells. Because fucose-specific lectins could block EcolCDT-II-mediated cell cycle arrest and CdtA and CdtC subunits could bind immobilized fucose, a sugar moiety containing fucose has been suggested as a likely component of the EcolCDT-II host cell receptor (McSweeney & Dreyfus, 2005). These findings are consistent with reduced binding of single amino acid AactCdtA subunit mutants to fucose-containing glycoprotein and correlation with reduced cytotoxicity of corresponding reconstituted AactCDT holotoxin for cultured Chinese hamster ovary (CHO) cells (Cao et al., 2004). However, this is difficult to reconcile with data suggesting that GM3 glycosphingolipid is the CDT host cell membrane receptor for intoxication of human monocyteic U937 cells by AactCdtC unless target cell membrane receptors vary between cells of different lineages (Mise et al., 2005). More recent studies examining the susceptibility of target cell lines expressing a wide range of surface membrane biomolecules to CDT representing each of the three distinct CdtA–CdtC sequence clusters described earlier suggest that a cell surface polypeptide component rather than glycoconjugates is the receptor that is most likely to be responsible for CDT–host cell specificity (Eshraghi et al., 2010). These findings are consistent with a requirement for a putative G protein-coupled transmembrane protein, designated TMEM181, which localizes to membrane lipid rafts in induction of EcolCdtB-I cytotoxicity by a novel loss-of-function haploid genetic screen using a highly sensitive myeloid leukaemia cell line (Carette et al., 2009). However, the possibility that TMEM181 is part of a complex that constitutes a functional receptor, that it plays a role in trafficking of a receptor–CdtB complex or that it represents a ligand unique to EcolCdtB-I cannot be ruled out. Nevertheless, the demonstration that the expression level of TMEM181 is rate limiting for intoxication of cell lines with differential sensitivity to CDT provides a molecular basis to explain the variable susceptibilities of cells from different lineages to intoxication. These apparent discrepancies between previously reported specificities might indicate the lack of a requirement for a shared molecular receptor among CDT produced by different bacterial pathogens. In support of this hypothesis is the observed differential receptor specificity of AactCDT/HducCDT, EcolCdtB-III and CjejCDT when compared against a battery of cell receptor molecular targets (Eshraghi et al., 2010). Since CDT-producing bacteria occupy different mucocutaneous niches and display diverse pathogenetic mechanisms, variable cell receptor requirement might indicate adaptation to the specificities of host cell targets.

Following receptor-mediated internalization in clathrin-coated pits, CdtB undergoes retrograde transport to the endoplasmic reticulum (ER) via the Golgi complex (Fig. 3; Cortes-Bratti et al., 2000; Guerra et al., 2005). The lack of protease inactivation or serum neutralization of HducCdtB and HducCdtC within minutes of cell surface binding suggests that conformational changes of cell surface-bound toxin are followed by rapid internalization. Consistent with this observation, irreversible inhibition of CHO cell proliferation occurs within 2 min of exposure to EcolCdtB-II (Aragon et al., 1997). Pharmacological, chemical or genetic disruption of clathrin-mediated endocytic pathways as well as Golgi complex transport abolish CDT cytotoxicity (Cortes-Bratti et al., 2000). Once taken up by mammalian cells, the catalytic subunit CdtB localizes to the nucleus presumably via alternate nuclear localization signals (NLSs; Lara-Tejero & Galán, 2000; McSweeney & Dreyfus, 2004; Nishikubo et al., 2003). An N-terminal NLS has been proposed for AactCdtB, while two C-terminal NLSs have been found in EcolCdtB-II (McSweeney & Dreyfus, 2004; Nishikubo et al., 2003). On the basis of highly conserved N-terminal amino acid sequences corresponding to the putative NLS of AactCdtB among all known bacterial CdtB orthologues, a modular structure consisting of an N-terminal domain responsible for nuclear transport and a C-terminal DNase-like domain capable of exerting DSBs has been proposed (McSweeney & Dreyfus, 2004; Nishikubo et al., 2003). Unlike the ‘A’ subunit of other AB toxins which generally translocates from the ER directly into the cell cytosol by a process of ER-associated degradation (ERAD), translocation of HducCdtB is ERAD-independent with the toxin subunit moving directly from the ER lumen to the nucleoplasm without unfolding (Guerra et al., 2009).

In S. Typhi, bacterial internalization into a Salmonella-containing vacuole of host cells leads to concurrent expression of StypCdtB, PllA and PllB which assemble into functional holotoxin complexes that are packaged into transport vesicles destined for extracellular secretion and intoxication of infected (autocrine) and uninfected neighbouring (paracrine) host cells (Fig. 4; Spanò et al., 2008). The absence of CDT receptor on infected host cells has been proposed as a protective mechanism against cytotoxicity allowing intracellular survival of S. Typhi, and thus persistence in infected host tissues (Spanò et al., 2008).
Nuclease and phosphatase activities of CdtB

The structural features of CdtB that predict a nuclease function are in agreement with demonstration of bacterial plasmid DNA digestion by recombinant EcolCdtB-II (Elwell & Dreyfus, 2000), AactCDT (Cao et al., 2005; Nishikubo et al., 2006) and HhepCdtB (Dassanayake et al., 2005a), and also with DNA DSBs in mammalian cells intoxicated with CjejCdtB (Lara-Tejero & Galaán, 2000) or HducCDT (Frisan et al., 2003). These observations have been extended to nuclear DNA fragmentation by transient expression, cellular transfection or nuclear microinjection of mammalian cells with CdtB alone (Frisan et al., 2003; Lara-Tejero & Galaán, 2000). Finally, rescue of a mouse B cell line from AactCDT-induced cytotoxic death with the DNA endonuclease inhibitor aurintricarboxylic acid further confirms the endonuclease-mediated DNA damage requirement for CdtB-mediated cellular toxicity (Ohguchi et al., 1998).

While it is well-established that cellular toxicity is mediated by the nuclease function of CdtB, other studies suggest that phosphatidylinositol-3,4,5-triphosphate (PIP₃) phosphatase activity plays a role in cellular toxicity (Shenker et al., 2007). This is based on in silico comparative analysis of predicted amino acid sequences suggesting that several phosphatases involved in cell cycle regulation and signal transduction, including inositol phosphatases, contain a protein fold similar to Mg²⁺-dependent endonucleases including mammalian DNase I and bacterial CDT (Dlakic, 2000). Demonstration of PIP₃ phosphatase activity is more clearly seen when using human leukemia T-cell lines with constitutively elevated PIP₃ levels because of mutations in SHIP1 and/or PTEN (Shenker et al., 2007). Therefore, an alternative mechanism of cellular toxicity might result from depletion of cell membrane PIP₃ and suppression of the protein kinase B (PKB)/Akt signalling pathway (Shenker et al., 2007). However, when compared with site-specific phosphatase-defective CdtB mutants or specific PIP₃ phosphatase inhibitors, the nuclease activity of AactCdtB alone was sufficient for induction of cell cycle arrest and apoptosis of a proliferating human monocytic U937 cell line (Rabin et al., 2009). Similarly, cell cycle arrest and death of haploid Saccharomyces (Sac.) cerevisiae yeast strains requires CdtB DNase I-like catalytic residues and nuclear localization (Matangkasombut et al., 2010). Since yeasts lack PIP₃, CdtB-induced DSB alone is sufficient to account for toxicity in this model (Matangkasombut et al., 2010). Taken together, these data suggest that the predominant mechanism of cellular toxicity varies according to target cell lineage with nuclease activity and DSB as the most prevalent mechanism, and PIP₃ phosphatase activity plays an accessory role in certain situations.

CDT-induced DNA repair response and cell cycle arrest

Mammalian cell cycle regulation is responsible for the proper maintenance of several physiological processes, including the maintenance of intact mucosal epithelial barriers and clonal expansion of lymphocyte subsets during adaptive immune response, both of which constitute important defence mechanisms against colonization and infection of mucocutaneous interfaces by bacterial pathogens (Oswald et al., 2005). Therefore, CDT-producing bacteria might have evolved a specialized mechanism to...
disrupt key mammalian cell functions in order to establish persistent colonization and cause disease in certain niches (Nougayrède et al., 2005; Oswald et al., 2005). On the basis of their ability to modulate the cell cycle and cause cell death, CDT has been classified as an inhibitory cyclomodulin (Nougayrède et al., 2005; Oswald et al., 2005). In fact, CDT was the first bacterial toxin shown to cause cell cycle arrest in mammalian cells (Tóth et al., 2009).

Cell cycle fidelity is maintained by redundant DNA damage checkpoint mechanisms, which are evolutionarily conserved signalling pathways that validate the integrity and accuracy of DNA replication at each phase of cell division (Bartek & Lukas, 2007; Jackson & Bartek, 2009). Activation of DNA damage checkpoints results in cell cycle arrest so that DNA can be repaired or, if damage is severe, progress to programmed cell death by apoptosis (Bartek & Lukas, 2007; Hoeijmakers, 2001). The DNA damage checkpoint network comprises upstream DNA damage sensors, signal transducers and downstream effectors (Bartek & Lukas, 2007). Specific pathways preferentially sense and respond to distinct types of DNA damage and initiate dedicated protective outputs (Hoeijmakers, 2001). Because DSBs are among the most severe DNA lesions, repair mechanisms involving error free homologous recombination and error-prone non-homologous end joining mechanisms will act synergistically to repair DSBs (Ciccia & Elledge, 2010). It is currently believed that CDT-induced DSB triggers a DDR similar to that caused by ionizing radiation (IR)-induced DSB and mediated by the phosphoinositide 3-kinase-related kinase ataxia telangiectasia mutated (ATM), encoded by the gene mutated in the rare autosomal recessive genetic disorder ataxia telangiectasia (AT; Fig. 5; Bartek & Lukas, 2007; Cortes-Bratti et al., 2001b; Derheimer & Kastan, 2010; Jackson & Bartek, 2009).

The ATM-dependent IR-induced DDR involves both induction of cell cycle arrest and initiation of DNA repair (Derheimer & Kastan, 2010). Major components of the ATM-dependent DNA damage signalling pathway include (i) the multifunctional MRN protein complex consisting of Mre11, Rad50 and Nbs1, (ii) histone H2AX, (iii) the cell cycle checkpoint regulator protein kinase Chk2 and (iv) the transcription factor p53. Downstream p53-induced activation of p21 and Chk2-mediated inactivation of cell division cycle 25 (CDC25) C phosphatase blocks cell cycle progression by inhibition of CDK–cyclin complexes and thus prevents entry into mitosis while promoting DNA repair.

stabilizing the DNA lesion and providing a platform for the binding of other DNA repair proteins (Derheimer & Kastan, 2010). Activated Chk2 reduces cyclin-dependent kinase (CDK) activity by several mechanisms including activation of the transcription factor p53 and subsequent induction of cyclin inhibitor p21 (Jackson & Bartek, 2009). CDKs are key regulators of the cell cycle and the inhibition of CDKs can result in arrest of the cell cycle progression at the G1/S, intra-S and G2/M checkpoints, allowing time for DNA repair before proceeding with replication or mitosis (Jackson & Bartek, 2009). Additionally, other ATM substrates have key roles in affecting cell cycle checkpoint function, such as Smc1 for intra-S phase, as reviewed by Derheimer & Kastan (2010).

The main evidence in support of CDT-induced DDR is based on demonstration of MRN and γH2AX activation shortly after exposure of susceptible cells to CDT. A large percentage of HeLa cells display γH2AX foci 2 h after HducCDT exposure, and nearly all cells are positive for γH2AX foci within 6–8 h post-exposure (Li et al., 2002). Both HducCDT and IR induce similar levels of Mre11 foci in HeLa cells and primary human dendritic cells (Li et al.,

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**Fig. 5.** Pathways of CDT-induced cell cycle arrest in mammalian cells. Following CDT-induced DNA double strand breaks, the ATM-mediated DNA damage response is initiated with subsequent activation of the multifunctional protein complex consisting of Mre11, Rad50 and Nbs1 (MRN), histone H2AX, the cell cycle regulator checkpoint kinase 2 (Chk2) and the transcription factor p53. Downstream p53-induced activation of p21 and Chk2-mediated inactivation of cell division cycle 25 (CDC25) C phosphatase blocks cell cycle progression by inhibition of CDK–cyclin complexes and thus prevents entry into mitosis while promoting DNA repair.
Nuclear microinjection of purified recombinant HducCdtB into HeLa cells induces Mre11 foci to the same extent as HducCDT holotoxin-treated cells within 1 h, suggesting that the active subunit CdtB is sufficient and necessary to elicit DDR (Li et al., 2002). Increased Rad50 foci and γH2AX are also seen in primary human fibroblasts treated with CjejCdtB (Hassane et al., 2003), in primary and established human endothelial cells treated with EcolCdtB-V (Bielaszewska et al., 2005) and cultured human intestinal epithelial H407 cells exposed to HhepCdtB (Liyanage et al., 2010). As expected, γH2AX is readily detected in immortalized wild-type human B lymphocyte lines after HducCdtB exposure, but it is absent in ATM-defective leukemic B cell lines obtained from AT patients (Li et al., 2002). Analysis of diploid Sacc. cerevisiae yeast strains has provided additional evidence in support of CDT-induced DDR. Sacc. cerevisiae strains that lack Mre11, Rad50 or Xrs2, the budding yeast repair proteins equivalent to the MRN complex of mammalian cells, or other proteins required for DSB repair such as Rad51 or Rad55, are exquisitely sensitive to CjejCdtB, suggesting a critical role for HR in repair of CDT-induced DNA damage (Kitagawa et al., 2007).

Other evidence in support of a CDT-induced DDR is based on demonstration of cell cycle checkpoint activation, which further confirms data obtained by monitoring DNA damage signalling. Human HL fibroblast and larynx carcinoma HEp-2 cell lines display similar cell cycle checkpoint response kinetics consisting of p53, Chk2 and Cdk1 phosphorylation and upregulation of p21 and p27, following exposure to IR or HducCdtB (Cortes-Bratti et al., 2001b). In common with IR, rat fibroblast cell lines exposed to HhepCdtB exhibit c-Myc and ATM-dependent activation of DNA damage checkpoint responses (Guerra et al., 2010). Even though this is not a genotoxin-specific phenomenon, formation of actin stress fibres through the ATM-dependent activation of small GTPase RhoA is seen in HeLa cells following HducCdt-induced DSBs (Frisan et al., 2003). Formation of actin stress fibres is also seen in Hep-2 cells exposed to HpuCdtD and CHO cells exposed to EcolCdtD-II (Aragon et al., 1997; Ceelen et al., 2006). More recently, the formation of actin stress fibres in HeLa cells exposed to HducCdtD was shown to result from the activation of nuclear RhoA-specific guanine nucleotide exchange factor (GEF) Net1 (Guerra et al., 2008).

Currently, CDT is known to cause ATM-dependent cell cycle arrest at the G2/M and G1/S transitions, although definitive molecular analysis could further strengthen these observations. The CDT-mediated G2/M arrest is a result, at least in part, of the activation of Chk2 by activated ATM upon sensing DSBs. Activated Chk2 phosphorylates and inactivates cell division cycle 25 (CDC25) C phosphatase. The resulting accumulation of phosphorylated cyclin B–CDK1 complex prevents mitotic entry (Ge et al., 2008; Smith & Bayles, 2006). The mechanism for CDT-mediated G1/S arrest is thought to be p53-dependent. Activated ATM phosphorylates p53, and the resulting upregulation of p21 inhibits cyclin E–CDK2, which blocks S-phase entry (Ge et al., 2008; Smith & Bayles, 2006). However, p53-independent upregulation of p21 following CDT treatment has been reported (Smith & Bayles, 2006). Although there is limited evidence supporting CDT-mediated intra-S-phase checkpoint activation in yeast, the potential contribution of this checkpoint has yet to be investigated in mammalian systems (Matangkasombut et al., 2010).

Limitations in our understanding of the molecular mechanisms of CDT-induced cell cycle arrest lie in the techniques that are commonly employed to address this question. With the exception of a few instances indicating accumulation of phosphorylated Cdk1 (Cdc2) prior to cell cycle arrest, CDT-induced arrest of the cell cycle relies primarily on demonstration of altered patterns of DNA staining with propidium iodide and analysis by fluorescence-activated cell sorting (Bielaszewska et al., 2005; Comayras et al., 1997; Cortes-Bratti et al., 2001b; Péres et al., 1997). At least two G2/M checkpoints exist in mammalian cells: an ATM-dependent transient checkpoint which activates in G2 phase cells immediately after DNA damage and a prolonged ATM-independent checkpoint which is activated several hours after damage and reflects accumulation of cells in the G2 phase that were initially damaged during S-phase (Weiss et al., 2003; Xu et al., 2002). Because CDT might induce arrest at multiple stages of the cell cycle, measurement of DNA content alone cannot distinguish between these two checkpoints, highlighting the need to delineate the mechanism of CDT-induced cell cycle arrest in greater detail.

**CDT-mediated apoptosis**

Apoptosis is a physiological mechanism of cell death present in multicellular organisms for the controlled elimination of unwanted cells. Similar to creating a block in the cell cycle, bacterial toxins can induce apoptosis of host cells to facilitate colonization, persistent infection and chronic disease by hampering healing (Fig. 6; Nougayrède et al., 2005; Ohara et al., 2011; Oswald et al., 2005). Diverse factors and stimuli can initiate signalling pathways leading to apoptotic cell death by two major mechanisms; the extrinsic pathway involving death receptor activation and the intrinsic (or mitochondrial) pathway that can be activated by several stimuli (environmental toxicants, drugs and toxins) that provoke cell stress or damage (Taylor et al., 2008). The extrinsic pathway is activated by the binding of extracellular death ligands (for example FasL and tumour necrosis factor α) to transmembrane death receptors (for example FAS), while the intrinsic pathway is activated by stimuli that alter the mitochondrial outer membrane permeability (Ow et al., 2008). In some cases, the extrinsic pathway can also be activated by certain DNA-damaging drugs (Roos & Kaina, 2006). The activation of the intrinsic pathway depends on the activation of one or more members of the BH3-only protein family including Bax, Bid, Puma and Noxa (Taylor et al., 2008). Accumulation of activated BH3-only proteins beyond a critical level
The mechanisms involved in CDT-induced apoptosis are incompletely understood and currently thought to be dependent on the particular type of target cell (Belibasakis et al., 2004; Dreyfus, 2003). Based on the observations of early DNA fragmentation and activation of DDR in several p53 wild-type and p53-defective cell lines, it is now clear that apoptosis is a downstream event of CdtB-induced DDR, which can be mediated through both p53-dependent and -independent pathways (Cortes-Bratti et al., 2001b;
Frisan et al., 2003; Liyanage et al., 2010; Ohara et al., 2004). Similar to other genotoxins, cells with wild-type p53 are more sensitive to CDT-induced apoptosis than p53-deficient cells. For example, in human leukaemia T-cell lines, caspases are activated earlier in p53 wild-type MOLT-4 cells (highly CDT sensitive) than they are in p53-mutated Jurkat cells (Cheng & Haas, 1990; Ohara et al., 2004).

The CDT produced by several bacteria can induce apoptosis in a broad range of cell types including proliferating and non-proliferating primary cells of fibroblastic (primary human fibroblasts; Wising et al., 2005), endothelial (human umbilical vein endothelial cells, Bielaszewska et al., 2005), haematopoietic origin (human peripheral blood mononuclear cells (HPBMC), Shenker et al., 2001; HPBMC, CD4+ T cells and CD14+ monocytes, Wising et al., 2005), and established cell lines of epithelial (H407, Liyanage et al., 2010; HeLa, Mooney et al., 2001; immortalized human gingival keratinocytes (HIGK), Alaoui-El-Azher et al., 2010; Ca9-22, Yamamoto et al., 2004; HEp-2, HeLa and HaCaT, Wising et al., 2005), endothelial (human EA.hy 926 and human brain microvascular endothelial cells, Bielaszewska et al., 2005), and haematopoietic origin (MOLT-4 and Jurkat, Ohara et al., 2004; 28SC, Hickey et al., 2005; TTHP-1, Wising et al., 2005) lineages (reviewed by Smith & Bayles, 2006). Similarly, caspase pathway activation following CDT intoxication has been documented in cultured epithelial (H407, Liyanage et al., 2010; HeLa, Wising et al., 2005; Ca9-22, Yamamoto et al., 2004; HIGK, Alaoui-El-Azher et al., 2010), endothelial (EA.hy 926, Bielaszewska et al., 2005) and haematopoietic (MOLT-4 and Jurkat, Ohara et al., 2004; 28SC, Hickey et al., 2005; HPBMC, Shenker et al., 2001; TTHP-1 and CD4+ T cells, Wising et al., 2005) lineages.

Recently, we reported that activation of the intrinsic (mitochondrial) apoptotic pathway follows DDAVPP treatment of human intestinal epithelial H407 cells (Liyanage et al., 2010). Sequential upregulation of Bax and downregulation of Bcl-2 led to cytochrome c release and the subsequent activation of caspase-9 within 5 h of exposure to DDAVPP (Liyanage et al., 2010). While the activation of caspase-9 peaked at 12 h, caspase-3 was not activated until 24 h after DDAVPP exposure, and the activity of caspase-8 did not change (Liyanage et al., 2010). Based on caspase activation patterns in this and other cell lines, and together with other experimental evidence, the major mechanism for CDT-induced apoptosis appears to be through the ATM-dependent intrinsic pathway (Fig. 6; Alaoui-El-Azher et al., 2010; Bielaszewska et al., 2005; Hickey et al., 2005; Ohara et al., 2004; Shenker et al., 2001; Yamamoto et al., 2004). In support of this hypothesis is a significant reduction of CDT-mediated apoptosis in human B-cell lines with overexpressed Bcl-2, supporting the suggestion of an intrinsic pathway-dependent mechanism of apoptosis activation by CDT (Ohguchi et al., 1998; Shenker et al., 2001). However, elevation of caspase-2 activity following AactCDT treatment of p53-mutated Jurkat cells suggests a direct caspase-2-mediated Bax/Bak translocation as the mechanism of intrinsic apoptotic pathway activation (Ohara et al., 2004). Although previous studies showed complete inhibition of AactCDT-induced apoptosis of human T-cell leukaemia Jurkat and MOLT-4 cell lines by the general caspase inhibitor z-VAD-fmk at 16 h (Ohara et al., 2004), failure of this inhibitor to completely rescue AactCDT-induced cell death at 24–48 h suggested a caspase-independent (late) cell death pathway (Ohara et al., 2008). This apparent late cell death in approximately 35% of treated cells has been attributed to intracellular accumulation of reactive oxygen species (Ohara et al., 2008). However, overexpression of Bcl-2 completely inhibited late cell death, suggesting that this pathway is also mediated through alterations in mitochondrial membrane permeability (Ohara et al., 2008). Finally, simultaneous activation of both intrinsic and extrinsic apoptotic pathways has also been suggested with human B lymphoblastoid JY and monocytic 28SC cell lines (Hickey et al., 2005; Shenker et al., 2001).

The role of ATM in CDT-mediated apoptosis is incompletely characterized. There is limited evidence suggesting CDT wild-type SN-B1 and JAC-B2 cells (Epstein–Barr virus-transformed B lymphocytes from healthy donors) are more susceptible to HducCDT-induced apoptosis compared with ATM-defective (lymphoblastoid cell lines from AT patients; Cortes-Bratti et al., 2001b). Based on caspase activation patterns, pharmacological inhibition of ATM or siRNA knock down of Chk2, it was recently shown that AactCDT-induced apoptosis of HIGK is mediated through the ATM-dependent DDR pathway (Alaoui-El-Azher et al., 2010).

CDT cellular specificity and susceptibility

It has been proposed that the effects of CDT are cell-type-dependent (Belibasakis et al., 2004; Dreyfus, 2003). Irrespective of the bacterial source of CDT and before characteristic nuclear and cytoplasmic distension are seen, cultured cell lines of epithelial and endothelial origins primarily arrest in the G2/M phase (Elwell et al., 2001; Lara-Tejero & Galán, 2001; Smith & Bayles, 2006; Whitehouse et al., 1998; Wising et al., 2005; Young et al., 2000b), whereas cells of fibroblastic origin arrest both in the G1/S and G2/M phases of the cell cycle (Belibasakis et al., 2004; Cortes-Bratti et al., 2001b; Hassane et al., 2003; Smith & Bayles, 2006; Wising et al., 2005). In contrast, cell lines of haematopoietic lineage including lymphocytes, monocytes, macrophages and dendritic cells, not only are several orders of magnitude more susceptible to CDT (pg versus μg as described below and in Fig. 7) but also display rapid apoptosis after a transient arrest of the cell cycle (Belibasakis et al., 2004; Cortes-Bratti et al., 2001b; Hassane et al., 2003; Smith & Bayles, 2006).

The lowest effective dose of recombinant CDT that can intoxicate cell lines of haematopoietic lineage varies between 10 and 50 pg ml⁻¹ compared with 1 and 5 μg ml⁻¹ for other cell types (Shenker et al., 2007). More than...
90% of either human Jurkat T cells or THP-1 monocytic cells undergo apoptosis within 24–48 h after treatment with 100 ng HducCDT ml\(^{-1}\), while only 30% of HeLa or HaCaT epithelial cells or primary human fibroblasts are intoxicated under the same conditions (Wising et al., 2005). Based on their extreme susceptibility, lymphocytes have been proposed as the \textit{in vivo} target of CDT, suggesting that immunomodulation is responsible for persistent bacterial colonization (Ge et al., 2005; Pratt et al., 2006; Shenker et al., 2007). Although CDT is a broad range genotoxin (Ge et al., 2005; Pratt et al., 2006; Shenker et al., 2007), it remains to be shown whether this apparent cell type specificity is in part attributable to inherent differences in cell surface receptor binding of CdtA and CdtC to host cell membrane based on: (i) host cell surface biomolecule chemical composition or density, (ii) intrinsic differences in CdtB uptake and nuclear translocation or (iii) variable target cell lineage DDR competence (Carette et al., 2009; Eshraghi et al., 2010). However, other factors that can determine the outcome of CDT interactions with susceptible cells, including differential receptor intrinsic affinity and also amino acid sequence divergence of CdtA and CdtC binding subunits encoded by different bacteria, need further detailed analysis (Carette et al., 2009; Eshraghi et al., 2010).

The mutational status of individual cell lines is another critical factor that can determine activation of specific checkpoint and apoptotic pathways, and thus the stage of cell cycle arrest and kinetics of progression to apoptosis in response to genotoxic injury. As described earlier, two of the most important mediators of CDT-induced DDR are ATM and p53, both of which play critical roles in determining the stage of cell cycle arrest and pathways and efficiency of apoptosis execution. ATM is required for the initiation of G1/S, intra-S and G2/M checkpoint arrest (Derheimer & Kastan, 2010; Xu et al., 2002). However, there are at least two distinct G2/M checkpoints, and only the rapid-transient checkpoint is ATM-dependent (Xu et al., 2002). Most established cell lines are tumour derived and frequently contain mutations in tumour suppressors, including p53 (Cheng & Haas, 1990). Similar to ATM, p53 is required for the initiation and maintenance of G1/S checkpoint and can play a role in arrest at the G2/M stage of the cell cycle (Giono & Manfredi, 2006). As described earlier, p53 wild-type cells are more susceptible to apoptosis compared with p53 defective cells (Roos & Kaina, 2006).

Since the cellular responses to CDT-induced damage are very similar to IR-induced DDR, the inherent differential tissue susceptibility to IR-induced DNA damage may also be involved in the pathogenesis of diseases caused by CDT-producing bacterial pathogens (Gudkov & Komarova, 2003; Smith & Bayles, 2006). Rapidly proliferating cells including haematopoietic and intestinal epithelial cells are the most radiosensitive cell types, while non-proliferating cells of the nervous, respiratory, urinary, endocrine, musculoskeletal and mesenchymal tissues are relatively radioresistant (Gudkov & Komarova, 2003). The rate of cell division does not always correlate with cellular radiosensitivity, as extremely radiosensitive adult thymus, spleen and bone marrow stem cells consist mostly of quiescent cells (Gudkov & Komarova, 2003). However, the expression levels of p53 and several p53-responsive pro-apoptotic genes including \textit{bax} and \textit{fas/ apo1} correlate with tissue radiosensitivity, where highly radiosensitive tissues have higher expression levels of p53 and p53-responsive pro-apoptotic genes (Gudkov & Komarova, 2003). As a general rule, haematopoietic cells undergo rapid apoptosis following IR exposure, whereas fibroblasts undergo permanent growth arrest and epithelial cells, with the exception of the intestinal epithelium which undergo rapid p53-mediated apoptosis, exhibit reversible arrest, while
p53-deficient cells from all lineages tend to display a brief arrest (Gudkov & Komarova, 2003).

In this context it is interesting to note that the CDT-induced G1/S cell cycle arrest in fibroblasts has been observed exclusively in primary cell lines, which are likely to have wild-type p53, and thus retain an intact G1/S checkpoint (Hassane et al., 2003). In contrast, G2/M cell cycle arrest does not depend on p53 for initiation, and therefore it is possible for established p53-deficient cell lines to retain a functional G2/M checkpoint (Giono & Manfredi, 2006). Indeed, the G2/M cell cycle arrest is the predominant type of cell cycle arrest associated with CDT toxicity (Smith & Bayles, 2006). At least in T-cell leukaemia cell lines, susceptibility to CDT-induced apoptosis is dependent on the p53 status of the cell line, as p53 wild-type cells are more susceptible to apoptosis compared with p53-deficient cells (Ohara et al., 2004).

The differential tissue susceptibility to IR mirrors the cell type susceptibility to CDT (haematopoietic lineage hypersusceptibility, growth arrest in epithelial, endothelial or fibroblast lineages and CDT resistance in p53-defective cells from all lineages). To our knowledge, epithelial cell lines primarily show G2/M phase cell cycle arrest and a slow progression to apoptosis which can take several hours to days after CDT treatment. Investigating the mechanism of CDT intoxication in primary intestinal epithelial cells should provide additional mechanistic insights into the pathogenesis of disease caused by CDT-producing bacterial pathogens.

It is conceivable that the CDT-induced DDR might be distinct from that induced by IR, yet some of the pathways may overlap. The ability of a cell line to generate DDR is also an important consideration which determines genotoxic susceptibility, particularly for agents that cause DSBs (Roos & Kaina, 2006). While DSBs can be repaired by either relatively error free homologous recombination (HR) or error-prone non-homologous end joining (NHEJ), the latter may lead to chromosomal rearrangements particularly in cells in which this mechanism is preferentially utilized, such as cells in G1 phase of the cell cycle (i.e. resting fibroblasts, hepatocytes) or in lymphocytes undergoing V(D)J recombination-associated DSB repair during clonal selection (Callén et al., 2007; Roos & Kaina, 2006). In HR-defective cells or cells that preferentially repair DSBs by NHEJ, the resulting chromosomal rearrangements can lead to apoptosis (Roos & Kaina, 2006). At least in Saccharomyces cerevisiae yeast, two types of mutations display hyper-sensitivity to AactCdtB: strains defective in sensing DNA strand breaks and strains with defective HR repair (Matangkasombut et al., 2010). Since yeasts use HR as their primary means of DSB repair, the data suggest that cells defective in DSBR repair are more sensitive to CdtB.

**Role of CDT in disease**

Naturally occurring infections with *Haem. ducreyi* and *A. actinomycetemcomitans* can elicit serum IgG antibodies to individual CdtABC protein subunits indicating development of a host adaptive immune response to toxin expressed during infection (Ando et al., 2010; Mbwana et al., 2003; Xynogala et al., 2009). It has been suggested that the absence of AactCdtC-specific IgG in sera of patients with localized aggressive periodontitis compared with those with generalized aggressive periodontitis might be attributable to differences either in CdtC expression by different strains or in the extent or duration of these infections in an individual patient (Ando et al., 2010). Serum antibodies that can neutralize the biological activity of CDT have been demonstrated in patients with chancroid (anti-HducCDT; Mbwana et al., 2003) and periodontitis (anti-AactCDT; Ando et al., 2010; Xynogala et al., 2009), and in patients who had recovered from campylobacteriosis (anti-CjejCDT; Abuoun et al., 2005). Moreover, development of high levels of CDT-neutralizing IgG in sera and genital tissues of mice immunized with HducCDT toxoid compared with native toxin suggests a potential vaccine application for CDT toxoid in protection against chancroid (Lundqvist et al., 2010). However, it remains to be determined whether serum IgG antibodies to individual CDT subunits can (i) prevent acute infection, (ii) contribute to recovery from active infection or (iii) protect against reinfection.

Aside from demonstrating an adaptive humoral immune response to CDT following natural infection or immunization, nearly all studies on the role of CDT in disease have focused on *in vitro* models of eukaryotic cell genotoxicity, and direct experimental evidence demonstrating the role of CDT in disease of human and animal hosts is limited (Ge et al., 2005; Smith & Bayles, 2006; Stevens et al., 1999). Failure to demonstrate differences in skin colonization and lesion development among human volunteers and rabbits inoculated with wild-type *Haem. ducreyi* compared with an isogenic strain with an inactivated *cdtC* gene suggests that CDT is not required for cutaneous infection with this pathogen (Stevens et al., 1999; Young et al., 2001). While *cdtA*-, *cdtB*-, and *cdtC*-negative mutant strains were no longer cytotoxic for cultured HeLa cells *in vitro*, a difference in the onset and degree of cutaneous changes elicited by mutant strains compared with the corresponding wild-type *Haem. ducreyi* parent strain in a rabbit model of chancroid could not be demonstrated, further suggesting that CDT does not contribute to the early stage of skin infection in this model (Lewis et al., 2001). Although a dose-dependent inflammatory response was seen following intradermal inoculation of rabbits with reconstituted recombinant HducCDT holotoxin, but not with individual subunits, incomplete characterization of the cellular infiltrate and mechanism of inflammation hinder interpretation of these observations (Wising et al., 2002). Similarly, intradermal inoculation of rabbits with an avirulent *Haem. ducreyi* strain together with 10 μg of reconstituted recombinant HducCDT holotoxin resulted in development of larger and ulcerated skin lesions when compared with the avirulent strain alone (Wising et al.,...
(2005). Considering that HducCDT is highly toxic to a variety of mammalian cells in vitro, it is conceivable that local cellular damage was responsible for the dermal response seen with purified toxin in the rabbit model. In the context of sexually transmitted disease associated with Haem. ducreyi, HducCDT might play a role in persistent infection and delayed healing which together can promote further disease transmission rather than initiation of mucocutaneous infection.

Preliminary in vivo evidence suggesting that CDT contributes to mucosal epithelial cell damage and altered healing response, which extends the earlier observations of Wising et al. (2002) in the rabbit model of chancroid ulcer, has recently been reported in a gingival rat model of AactCDT (Ohara et al., 2011). In this model, oral sulcular epithelial cell degeneration and sloughing accompanied by local arrest of epithelial cell regenerative response was found within 3 days after in situ inoculation of oral gingival sulci of rats with purified parent AactCDT holotoxin expressed in E. coli, but not with AactCDT holotoxin reconstituted with a mutated CdtB DNase I-like H274A catalytic site (Ohara et al., 2011).

Since CDT is produced by several intestinal bacterial pathogens, early studies examined the role of purified CDT and mutant strains in the pathogenesis of diarrhoeal disease. Development of profuse watery diarrhoea accompanied with intestinal fluid accumulation and colonic epithelial cell damage within 12 h after intragastric inoculation of conventional suckling mice with purified SdysCDT expressed in E. coli suggested a role for CDT in diarrhoeal disease associated with Shigella dysenteriae infection (Okuda et al., 1997). These observations were further supported by demonstration of impaired transcription of C. jejuni CDT mutant compared with the isogenic wild-type parent strain across the intestinal epithelial barrier of adult SCID mice at 2 h, but not at 6 or 24 h after intragastric inoculation with 105 c.f.u. bacteria (Purdy et al., 2000). Although the data suggest a potential role for CDT in direct intestinal epithelial cell damage and disease, intragastric administration of massive doses of toxin or bacteria, respectively, in immature and immunocompromised hosts might not be representative of the natural disease. Therefore, it is still unclear whether CDT contributes to intestinal epithelial barrier dysfunction and systemic spread of CDT-producing bacteria.

Because HhepCDT is the only known virulence factor found in Helicobacter (Hel.) hepaticus, a host-adapted pathogen of mice (Stuerbaum et al., 2003), laboratory mice have been used as a model to uncover pathogenetic mechanisms associated with CDT in infection and disease. Studies in laboratory mice, mostly with Hel. hepaticus (Ge et al., 2005; Pratt et al., 2006; Young et al., 2004), but also with Hel. cinaedi (Shen et al., 2009) and C. jejuni (Fox et al., 2004a) and others (reviewed by Ge et al., 2008) suggest that CDT potentially contributes to bacterial virulence. On the basis of a more rapid intestinal clearance of Hel. hepaticus CdtB-negative mutants compared with the wild-type parent strain in orally inoculated conventional and IL-10−/− deficient mice, a role for HhepCDT in resistance against host defence mechanisms has been suggested (Ge et al., 2005; Pratt et al., 2006). The lack of serum IgG1 and significantly lower IgG2c responses to Hel. hepaticus in IL-10−/− mice infected with a Hel. hepaticus CdtB-negative mutant compared with mice infected with wild-type Hel. hepaticus up to 8 months post-inoculation suggest an immunomodulatory role for HhepCDT in this model (Pratt et al., 2006). In these studies, IL-10−/− mice that recovered from infection with the CdtB-negative mutant strain were partially protected against subsequent challenge with either the mutant or wild-type Hel. hepaticus strains (Pratt et al., 2006). However, because protection of mice recovered from infection with wild-type Hel. hepaticus was not evaluated, it is unknown whether partial protection is a function of a lack of exposure to CDT or a characteristic of Hel. hepaticus infection in IL-10−/− mice. In other studies using highly susceptible A/JcR male mice, the prevalence and level of caecal colonization by a Hel. hepaticus CdtB-negative mutant were reduced compared with the wild-type parent strain at 4 and 10 months post-inoculation, whereas hepatic colonization levels were similar for both strains at 4 months, but reduced at 10 months post-inoculation in mice inoculated with the mutant strain (Ge et al., 2007). Either strain produced similar degrees of hepatic inflammation early after infection, but only mice infected with the wild-type strain progressed to develop dysplastic changes by 10 months post-inoculation (Ge et al., 2007). Taken together, these studies demonstrate a role for HhepCDT in modulating host adaptive immune response so that persistent intestinal colonization leads to systemic translocation of bacteria and localization to the liver where chronic infection can result in development of hepatocellular carcinoma. More recently, infection of immunodeficient mice engrafted with human haematopoietic stem and progenitor cells with an S. Typhi mutant strain lacking PltB suggested a role for typhoid toxin in persistent infection (Song et al., 2010). However, none of the mice developed clinical disease in this model. Future studies with laboratory mice should uncover more precisely the stage in pathogenesis of disease and the specific cellular targets of CDT that are responsible for persistent infection and development of disease.

High concentrations of all three CjejCDT subunits are present in detergent extracts of purified outer membrane preparations of C. jejuni, suggesting that it is primarily membrane-associated (Hickey et al., 2000; Lindmark et al., 2009). Incubation of C. jejuni in the presence of 25 mM or 0.1 % bile acid sodium deoxycholate, a concentration physiologically relevant to the intestinal lumen, releases the membrane-associated CdtA, CdtB and CdtC subunits into the culture supernatant (Hickey et al., 2005). Since CjejCDT holotoxin can elicit established human intestinal epithelial cell lines to produce CXCL8, a potent pro-inflammatory chemokine responsible for recruitment of
polymorphonuclear neutrophils (PMNs) in the intestinal mucosa, a role for CDT in initiation of host innate defence has been suggested (Hickey et al., 2000; Konkel et al., 2001; Murphy et al., 2011; Young et al., 2007). However, since PMNs are expected to eliminate C. jejuni, the benefit of eliciting a pro-inflammatory response seems less desirable if bacterial infection is prevented. Given that massive translocation of PMNs across the intestinal epithelium, as seen in the initial stages of campylobacteriosis, results in increased intestinal permeability and leakage of extracellular fluid into the gut lumen (Blikslager et al., 2007; Dasti et al., 2010), alterations in the gut microenvironment might indirectly promote local expansion of C. jejuni, thus allowing increased shedding of bacteria in faeces, contamination of the environment and further spread of the disease to susceptible hosts.

Development of a host adaptive immune response to an individual CDT protein subunit is seen following spontaneous infection and disease caused by CDT-producing A. actinomycetemcomitans, C. jejuni and Haem. ducreyi. However, experimental infections with Hel. hepaticus in a laboratory mouse model clearly show that CDT can modulate both the level and isotype antibody response of the host and establish persistent infection. Future studies should define which bacterial clearance mechanism is specifically targeted by CDT and its relationship to host immune response modulation.

Conclusions and future directions

Since the discovery of CDT in 1987, our understanding of the ecology of CDT-producing bacterial species has greatly expanded and the molecular biology of CDT-induced cellular genotoxicity has improved significantly. However, the natural history and contribution of CDT produced by individual bacterial pathogens in the context of mucocutaneous colonization, initiation and persistence of infection and disease in their respective host niches remain incompletely understood. Of paramount importance is the production of CDT by all major food- and water-borne pathogens, including several pathotypes of E. coli and species of Campylobacter, Shigella and Salmonella. Greater than 85% prevalence of the CDT gene among clinical isolates of CDT-producing species of the families Pasteurellaceae, Campylobacteraeae and Helicobacteriaceae suggests a critical role for this toxin in the pathogenesis of diseases caused by members of these families. In contrast, less than 14% prevalence of CDT gene carriage together with evidence of HGT in clinical isolates of the enterobacteriaceae implies a conditional requirement for maintenance of this toxin in certain members of this family. Further studies on the ecology of CDT are clearly needed in order to clarify the significance of this toxin in different bacterial pathogens affecting a range of host species, including human beings.

Currently, CDT is the only member of a novel class of AB-type bacterial toxins that displays nuclease activity and translocates to the nucleus of a broad range of mammalian cell lineages where it exerts genotoxic damage resulting in cell cycle arrest and apoptosis. Further studies are needed in order to more precisely establish the relative importance of nuclease and phosphatase activities in disease pathogenesis. While the role of CDT-producing bacterial pathogens in orchestrating host adaptive immune response leading to persistent infection, inflammation and chronic disease such as periodontitis, chancroid, enterocolitis and hepatitis is relatively well documented, the underlying mechanisms, particularly in the context of infection and chronic disease associated with persistence and delayed healing, are incompletely understood. Given the availability of genetically engineered mouse models, determining the cellular targets of CDT in relation to specific stages of the disease process and host immune response modulation has the potential to expand our understanding of the role of this toxin in host–pathogen interactions. CDT is the only known virulence factor produced by Hel. hepaticus and Helicobacter bilis, and chronically infected mice develop inflammation-associated hepatic and colon cancer (Ericsson et al., 2010; Fox et al., 2011). Given the association of these and other CDT-producing bacterial pathogens with cancer and the ability of CDT to cause DNA damage in a wide range of mammalian cell lineages, a largely unexplored and provocative area of future research will be to determine whether or not CDT-producing bacteria can act as cofactors in promoting cancer development in their respective host niches. We hope that this review will generate interest among our colleagues and stimulate the next generation of microbiologists to tackle this evolving field of CDT-mediated microbial pathogenesis.

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References


kinase dephosphorylation and activation. *Infect Immun* 65, 5088–5095.


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Cytolethal distending toxin


