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Cytolethal Distending Toxin: A Bacterial Bullet Targeted to Nucleus

Masaru Ohara[1], Eric Oswald[2] and Motoyuki Sugai[1,*]

[1]Department of Bacteriology, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553; and [2]UMR1225 Institut National de la Recherche Agronomique-Ecole Nationale Veterinaire de Toulouse, France

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Cytolethal distending toxin (Cdt) is a newly added member of bacterial protein toxins that hijack the control system of eukaryotic cells. Cdts are produced by several pathogenic bacteria causing chronic infectious diseases. They are composed of three subunits, CdtA, CdtB and CdtC, which together form a ternary complex. CdtB is the active component, and CdtA and CdtC are involved in delivering the CdtB into the cells. The sophisticated strategy of Cdt to control host cells is CdtB-mediated limited DNA damage of the host cell chromosome, which triggers the response of the cell cycle checkpoint and results in G2 arrest in the cells. Cdt also induces apoptotic cell death of lymphocytes, which may be relevant to onset or persistence of chronic infection by the producing bacteria. The study of this toxin is expected to provide us information on a novel strategy by which bacteria interact with host cells.

Key words: apoptosis, cell cycle arrest, crystal structure, cytolethal distending toxin, nuclear transport.

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Isolation of Cdt and its encoded genes

Cytolethal distending toxin (Cdt) was discovered as a new heat-labile toxin from *Escherichia coli* and *Campylobacter* spp. associated with diarrheal disease (*1*, *2*). The term Cdt reflected the unique properties of this protein causing progressive cell distension and cytotoxicity to cultured cells such as CHO, HeLa, and Hep-2 cells (Fig. 1). Another unique phenotype of the Cdt-intoxicated cells is cell cycle arrest at the transition from G2 to M phase (Fig. 1). The genes of this unique toxin have been isolated as *cdt*A, -B, and -C, tandemly located in the *cdt* locus. A valiant *cdt* gene, *cdt*I-V, was found in *E. coli* (*3–6*). Full sets of *cdt* genes were also isolated from causative agents of chronic infection such as *Campylobacter jejuni* (*7*), *Haemophilus ducreyi* (*8*), *Shigella dysenteriae* (*9*), *Actinobacillus actinomycetemcomitans* (*10–12*), *Helicobacter hepaticus* (*13*), and other species (*14*, *15*). Recently, only the *cdtB* gene was discovered in the genome of *Salmonella typhi*, the causative bacteria of typhoid fever (*16*).

Complex formation

At the beginning of the Cdt study, it was uncertain which of the subunits were active and whether the three subunits formed a complex. Most recent studies indicate that all three components, CdtA, -B, and -C, are required for full activity (*17–21*). Pull-down assay revealed that the active Cdt holotoxin is a heterotrimer, consisting of CdtB as the enzymatically active subunit, and CdtA and CdtC which mediate the delivery of CdtB into host cells (*17–20*). The crystal structure of Cdt holotoxin was solved as a tripartite complex of CdtA, CdtB and CdtC, which will be discussed in later paragraph. *S. typhi* CdtB was reported to be an exceptional case of Cdt holotoxin. The genome sequence of *S. typhi* includes no apparent homologues of *cdtA* and *cdtC* in the vicinity of *cdtB*, but Cdt-induced intoxication occurs by the delivery of CdtB into the host cells during or after cellular internalization of the bacteria (*16*).

Active subunit, CdtB

It has been suggested that CdtB subunit is a nuclease or a phosphatase. Position-specific iterated (PSI) BLAST search of the protein data bank using CdtB polypeptides as query sequences indicated that the residues of DNase I involved in phosphodiester bond hydrolysis (His134 and His252) are conserved in CdtB as well as their respective hydrogen bond pairs (Glu78 and Asp212) in the *cdt*II genes (*22–24*). CdtB also contains a pentapeptide motif found in all DNase I enzymes. A crude Cdt preparation showed in vitro DNase activity against plasmid DNA as a substrate. Four of five proteins carrying mutations in amino acids corresponding to DNase I active-site residues lacked DNase activity in vitro and failed to induce cell cycle arrest and cytodistension as a holotoxin *in vivo* (*22*). Electroporation of CdtB directly into cells also resulted in cellular distension and chromatin fragmentation (*23*). Moreover, transient expression of the *C. jejuni cdtB* gene in cultured cells caused marked chromatin disruption (*25*). Microinjection of low amounts (0.001 mg/ml) of CdtB induced cell cycle arrest and cytodistension. Since *H. ducreyi* CdtB has 10^6 times lower activity for chromatin change than a bovine DNase I, a DNA repair complex such as that of histone H2AX and Rad50 was used as marker for the detection of limited double-strand breakage (*26*). *H. ducreyi* CdtB was demonstrated to induce histone H2AX phosphorylation and Mre11 relocalization as early as 1 h after intoxication of HeLa cells (*26*). *C. jejuni* Cdt was also shown to promote the formation of Rad50 foci, one of the DNA repair responses, which are formed around double-strand DNA breaks

*To whom correspondence should be addressed. Phone+81 82 257 5635, Fax: +81 82 257 5639, E-mail: sugai@hiroshima-u.ac.jp

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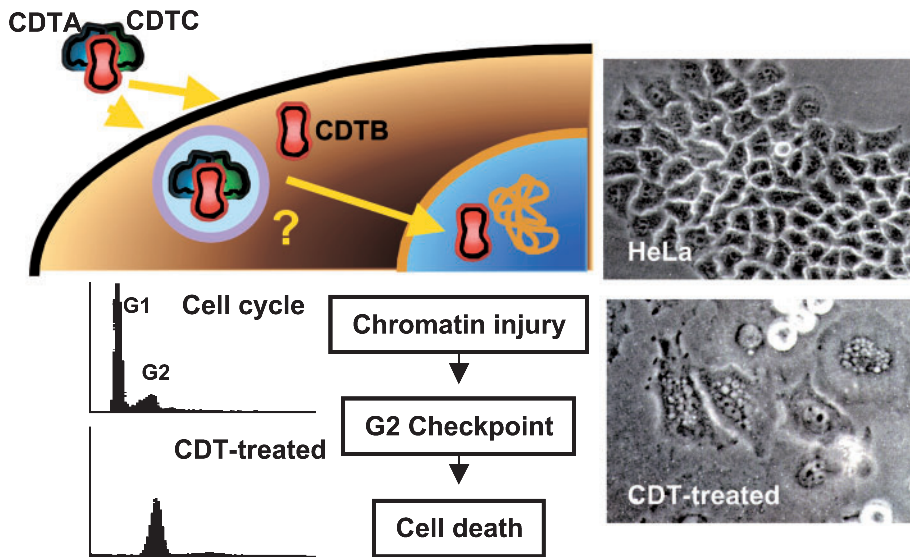

Fig. 1. **Cell cycle inhibitory effect of CDT.** Intoxicated HeLa cells with Cdt (10 pg/ml) shows G2 arrest after 24 h and progressive cellular distension after 48 h. The diagram at top left indicates the possible transport pathway of Cdt into the target nucleus. It is still not clear whether the whole Cdt complex or only the active subunit, CdtB enter the cell. Finally CdtB (or Cdt complex) enters the nucleus by active transport. In the nucleus, CDTB-mediated chromatin injury can trigger the activation of the checkpoint machinery and subsequent cell-death pathway.

(*27*). These findings suggest that the trigger of cellular response induced by Cdt is chromatin injury or double-strand breakage by the CdtB subunit.

Activation of G2 checkpoint

E. coli, *C. jejuni* and *H. ducreyi* Cdts were demonstrated to block the HeLa cell cycle at the G2/M transition by preventing dephosphrylation of the inactive, tyrosine-phosphorylated form of Cdc2 (*28–32*), suggesting that the Cdts from various bacteria activate some kinase/phosphatase in the signaling network controlling Cdc2 activity. Involvement of Cdc2 in the G2 checkpoint was also demonstrated in a yeast model, in which the Cdt-toxicity did not occur in CDK1 mutated yeast (*33*). Highly expressed Cdc25C prevents the Cdt-intoxicated cells from G2 arrest (*34*), suggesting that Cdc25C functions upstream of the signaling network to keep Cdc2 in inactivated form. *H. ducreyi* Cdt was found to activate the chk2 or 1 kinase in epithelial cells. This effect resembled the checkpoint response activated by ionizing radiation (*35*). Moreover, Cdt-intoxication was delayed in the Ataxia Telangiectasia-mutated gene (ATM)-deficient lymphoblastoid cell lines, suggesting that the response to Cdt is ATM-dependent. The ATM-dependent signaling pathway was further suggested by the finding that caffeine partly overrode Cdt-induced cell cycle arrest (*35*). The possible scenario of Cdt-induced G2 arrest is shown in Fig. 2.

Related molecules to Cdt-induced cell cycle arrest

H. ducreyi Cdt-treated fibroblasts showed an early induction of the cycline-dependent kinase inhibitor, p21 (Cip1/Waf1) (*35*). *A. actinomycetemcomitans* Cdt also induced G2 arrest in HS-72 cells, a murine B cell hybridoma, and up-regulated the expression of the p21 (*36*). In addition to p21, normal fibroblasts in which cell cycle was arrested both in G1 and G2 by *H. ducreyi* Cdt-treatment, showed an early induction of the P53 gene and an increased amount of phosphorylated p53 at the 15th serine residue (*35*). An ATM-deficient lymphoblastoid cell line treated with Cdt showed slower kinetics of p53 stabilization, resulting in slow intoxication by Cdt. p53 is mainly involved in the regulation of G1 checkpoint through transcriptional induction of p21 (*37*). It is possible that P53 is also involved in G2 checkpoint (*38*), but ectopic expression of a dominant negative P53 mutant did not inhibit Cdt-mediated G2 arrest in HS-72 B cell (*36*). Cdt-intoxicated endothelial cells were arrested in G2 without p53-phosphorylation (*39*). The role of p53 in Cdt-intoxication remains to be clarified in future studies.

Cdt transport

Cdt secreted from bacteria targets the host cells. *C. jejuni* CdtA and CdtC were shown to exist in bound form on the cell surface of HeLa cells (*57*). Also the incubation of HeLa cells with *H. ducreyi* CdtA-CdtC complex blocked the killing of these cells by the Cdt holotoxin (*58*). Intoxication of *H. ducreyi* Cdt was inhibited by removal of clathrin coat via K^+ depletion, suggesting that Cdt can be internalized through a clathrin-coated pit (*59*). Furthermore, intoxication was also inhibited in the cells treated with bafilomycin A1 or nocodazole, which are known to block the fusion of early endosomes with downstream compartments. Disruption of the Golgi complex by treatment with brefeldinA or ilimaquinone blocked intoxication, suggesting that Cdt can be transported by a retrograde pathway (*59*).

Since CdtB is implicated in a genotoxic role of Cdt, several attempts were made to probe CdtB in the target nucleus. CdtB microinjected into the cytoplasm was shown to localize in the nucleus and induce chromatin collapse (*25*, *60*). An *in vitro* transport assay demonstrated that the nuclear localization of CdtB is mediated by active transport (*60*). An assay using transient expression of a series of truncated CdtB-GFP fusion proteins revealed that residues 48–124 constitute the minimum region involved in nuclear transport of *A. actinomycetemcomitans* CdtB. Separately *E. coli* CdtB-II was demonstrated to possess nuclear localization signals (NLS) at 195–210 and 253–269 in the C-terminus of the molecule, which correspond to the bipartite NLS (*61*). These are the first demonstrations that a bacterial toxin possessing a unique domain for nuclear transport is transferred to the animal cell nucleus.

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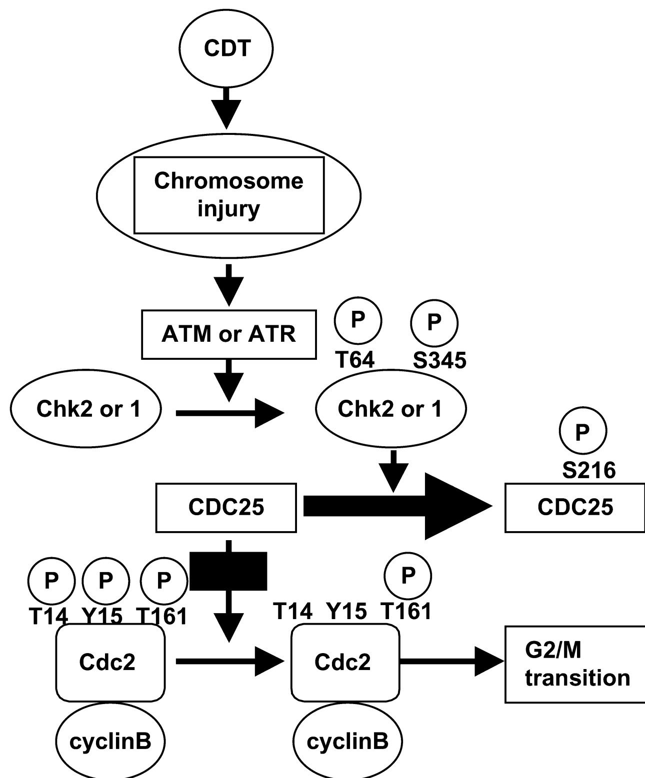

Fig. 2. **CDT-induced G2 checkpoint.** DNA damage by CdtB action could activate checkpoint control. In normal cells, dephosphorylation of threonine14 and tyrosine15 in Cdc2/cyclin B complex, a key molecule for the progression of the cell cycle, triggers G2/M transition in the cell cycle. However, in Cdt-intoxicated cells, these residues in Cdc2 remain phosphorylated in Cdc2/cyclin B complex, because of the Cdc25C-sequestration, induced by G2 checkpoint machinery.

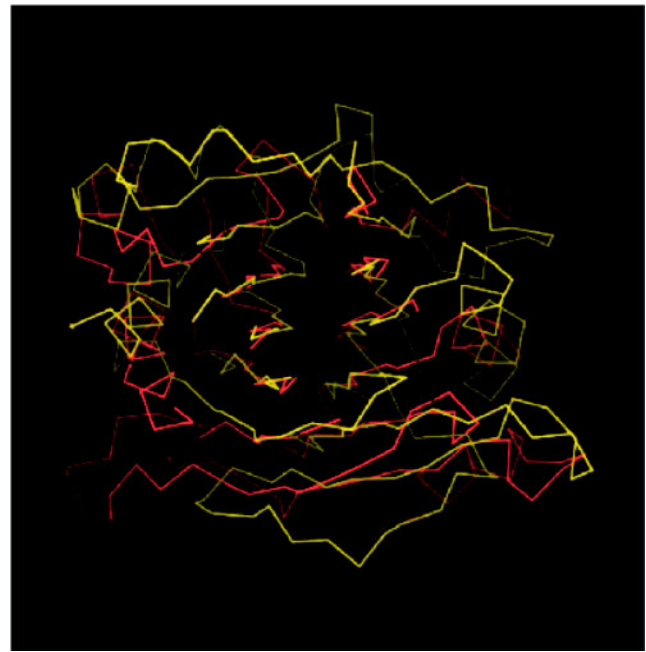

Fig. 3. **Structural alignment of DNase I to *Haemophilus ducreyi* CdtB.** DNase I and CdtB are shown in red and yellow respectively.

Crystal structure

The crystal structure of *H. ducreyi* Cdt holotoxin has recently been reported (*62*). Cdt forms a ternary complex of CdtA, CdtB, and CdtC with three interdependent molecular interfaces. The crystal structure of the CdtB subunit in the holotoxin was very similar to that of the DNase-I (Fig. 3), and CdtA and CdtC constitute two ricin-like lectin domains. In structural alignments with DNase-I, two critical histidine residues of CdtB (His160, and His274) coincide almost perfectly with the active-site histidine residues. Furthermore, highly conserved residues in DNase-I that contact with substrate DNA (Arg111, Asn170, and Arg41) have nearly identical counterparts in CdtB as Arg144, Asn201, and Arg117, respectively. However, dissimilarity is also found in some points. One of two possible catalytic histidine residues in CdtB does not have a hydrogen bond counterpart, due to the presence of a valine residue instead of a glutamic acid residue.

Cdt-induced cell death

Whole cells or culture supernatant of a haemolysin-deficient mutant of *H. ducreyi* induced apoptosis in Jurkat T cells, which was inhibited by CdtC-neutralizing antibodies (*40*). *A. actinomycetemcomitans* Cdt induces apoptosis not only in human T cells but also in B cell lines (*35*, *41–43*). That most lymphocytes and related cells are demonstrated to undergo apoptosis by Cdt suggests the immunosuppressive role of Cdt in the process of chronic infection (*12*, *35*, *44*). On the other hand *A. actinomycetemcomitans* Cdt does not induce apoptosis in human periodontal ligament cells or gingival fibroblasts, although DNA synthesis was inhibited in the early response (*45*). Thus, the molecular mechanism by which Cdt induces cell death of epithelial and fibroblastic cells remains unclear.

Cdt and diseases

Cdt was found from clinically isolated enteropathogens, such as *E. coli* and *C. jejuni*, which cause diarrhea in humans and animals (*1*, *2*). In addition the *cdt* genes were found to co-exist with other virulence genes such as *cnf* or *eae* (*46*, *47*). Indeed Cdt-producing *E. coli* induced excretion of watery feces in a suckling mouse model (*9*). However, many statistical studies showed that Cdt or the *cdt* gene was present in the bacteria isolated from the patients with diarrhea at relatively low frequency (*48*). Calves inoculated with a *cdt*-III negative mutant developed diarrhea in the same way as those inoculated with wild-type necrotoxic *E. coli* (NTEC2), which carries *cnf*2 and *cdt*-III genes on the pVir plasmid (*49*). Thus it is still unclear whether Cdt plays a role in causing diarrhea.

Other diseases in which Cdt was implicated in the pathogenesis are chancroid, a sexually transmitted genital ulcerative lesion (*8*, *30*, *50*) and periodontitis, a chronic inflammatory disease that is characterized by progressive destruction of the alveolar bone and eventual loss of the teeth (*51*). *A. actinomycetemcomitans* is one of the causative bacteria of the localized juvenile or adult periodontitis (*52*). Statistical analysis suggests that the cytotoxic and immunosuppressive properties of *A. actino-*

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mycetemcomitans Cdt may perturb the host immunity and contribute to the pathogenesis of aggressive periodontitis (*50*, *53–56*).

Epilogue

Several bacterial virulence factors have been shown to inhibit the proliferation of the target cells, suggesting the existence of bacterial mechanisms to control the eukaryotic cell cycle (*32*, *63*, *64*). Cdt is a representative example of toxins that hijack the control system in eukaryotic cells, and such virulence factors have been termed "cyclostatins" or "cyclomodulins" (*63*). The sophisticated strategy of the Cdt-producing pathogens to control host cells is CdtB-mediated limited DNA damage, which triggers the response that eventually halts the cell cycle (*25*, *65*). In addition, once in the target cells, CdtB sets out on a journey to the nucleus by using the host cell nuclear transport machinery (*60*, *61*). Further studies of such an intelligent bacterial weapon will surely provide more information at molecular level about the cunning strategies employed by pathogenic bacteria to achieve chronic infection.

REFERENCES

1. Johnson, W.M. and Lior, H. (1988) A new heat-labile cytolethal distending toxin (CLDT) produced by *Campylobacter* spp. *Microb. Pathog.* **4**, 115–126
2. Johnson, W.M. and Lior, H. (1988) A new heat-labile cytolethal distending toxin (CLDT) produced by *Escherichia coli* isolates from clinical material. *Microb. Pathog.* **4**, 103–113
3. Pickett, C.L., Cottle, D.L., Pesci, E.C., and Bikah, G. (1994) Cloning, sequencing, and expression of the *Escherichia coli* cytolethal distending toxin genes. *Infect. Immun.* **62**, 1046–1051
4. Scott, D.A. and Kaper, J.B. (1994) Cloning and sequencing of the genes encoding *Escherichia coli* cytolethal distending toxin. *Infect. Immun.* **62**, 244–251
5. Pérès, S.Y., Marches, O., Daigle, F., Nougayrede, J.P., Herault, F., Tasca, C., De Rycke, J., and Oswald, E. (1997) A new cytolethal distending toxin (CDT) from *Escherichia coli* producing CNF2 blocks HeLa cell division in G2/M phase. *Mol. Microbiol.* **24**, 1095–1107
6. Toth, I., Herault, F., Beutin, L., and Oswald, E. (2003) Production of cytolethal distending toxins by pathogenic *Escherichia coli* strains isolated from human and animal sources: establishment of the existence of a new *cdt* variant (Type IV). *J. Clin. Microbiol.* **41**, 4285–4291
7. Pickett, C.L., Pesci, E.C., Cottle, D.L., Russell, G., Erdem, A.N., and Zeytin, H. (1996) Prevalence of cytolethal distending toxin production in *Campylobacter jejuni* and relatedness of *Campylobacter* sp. *cdtB* gene. *Infect. Immun.* **64**, 2070–2078
8. Cope, L.D., Lumbley, S., Latimer, J.L., Klesney-Tait, J., Stevens, M.K., Johnson, L.S., Purven, M., Munson , R.S.J., Lagergård, T., Radolf, J.D., *et al.* (1997) A diffusible cytotoxin of *Haemophilus ducreyi*. *Proc. Natl. Acad. Sci. USA* **94**, 4056–4061
9. Okuda, J., Fukumoto, M., Takeda, Y., and Nishibuchi, M. (1997) Examination of diarrheagenicity of cytolethal distending toxin: suckling mouse response to the products of the *cdtABC* genes of *Shigella dysenteriae*. *Infect. Immun.* **65**, 428–433
10. Sugai, M., Kawamoto, T., Pérès, S.Y., Ueno, Y., Komatsuzawa, H., Fujiwara, T., Kurihara, H., Suginaka, H., and Oswald, E. (1998) The cell cycle-specific growth-inhibitory factor produced by *Actinobacillus actinomycetemcomitans* is a cytolethal distending toxin. *Infect. Immun.* **66**, 5008–5019
11. Mayer, M.P., Bueno, L.C., Hansen, E.J., and DiRienzo, J.M. (1999) Identification of a cytolethal distending toxin gene locus and features of a virulence-associated region in *Actinobacillus actinomycetemcomitans*. *Infect. Immun.* **67**, 1227–1237
12. Shenker, B.J., McKay, T., Datar, S., Miller, M., Chowhan, R., and Demuth, D. (1999) *Actinobacillus actinomycetemcomitans* immunosuppressive protein is a member of the family of cytolethal distending toxins capable of causing a G2 arrest in human T cells. *J. Immunol.* **162**, 4773–4780
13. Young, V.B., Knox, K.A., and Schauer, D.B. (2000) Cytolethal distending toxin sequence and activity in the enterohepatic pathogen *Helicobacter hepaticus*. *Infect. Immun.* **68**, 184–191
14. Mooney, A., Clyne, M., Curran, T., Doherty, D., Kilmartin, B., and Bourke, B. (2001) *Campylobacter upsaliensis* exerts a cytolethal distending toxin effect on HeLa cells and T lymphocytes. *Microbiology* **147**, 735–743
15. Kostia, S., Veijalainen, P., Hirvi, U., and Hanninen, M.L. (2003) Cytolethal distending toxin B gene (*cdtB*) homologues in taxa 2, 3 and 8 and in six canine isolates of *Helicobacter* sp. *flexispira*. *J. Med. Microbiol.* **52**, 103–108
16. Haghjoo, E. and Galan, J.E. (2004) *Salmonella typhi* encodes a functional cytolethal distending toxin that is delivered into host cells by a bacterial-internalization pathway. *Proc. Natl Acad. Sci. USA* **101**, 4614–4619
17. Lara-Tejero, M. and Galan, J.E. (2001) CdtA, CdtB, and CdtC form a tripartite complex that is required for cytolethal distending toxin activity. *Infect. Immun.* **69**, 4358–4365
18. Saiki, K., Konishi, K., Gomi, T., Nishihara, T., and Yoshikawa, M. (2001) Reconstitution and purification of cytolethal distending toxin of *Actinobacillus actinomycetemcomitans*. *Microbiol. Immunol.* **45**, 497–506
19. Wising, C., Svensson, L.A., Ahmed, H.J., Sundaeus, V., Ahlman, K., Jonsson, I.M., Molne, L., and Lagergard, T. (2002) Toxicity and immunogenicity of purified *Haemophilus ducreyi* cytolethal distending toxin in a rabbit model. *Microb. Pathog.* **33**, 49–62
20. Shenker, B.J., Besack, D., McKay, T., Pankoski, L., Zekavat, A., and Demuth, D.R. (2004) *Actinobacillus actinomycetemcomitans* cytolethal distending toxin (Cdt): evidence that the holotoxin is composed of three subunits: CdtA, CdtB, and CdtC. *J. Immunol.* **172**, 410–417
21. Avenaud, P., Castroviejo, M., Claret, S., Rosenbaum, J., Megraud, F., and Menard, A. (2004) Expression and activity of the cytolethal distending toxin of *Helicobacter hepaticus*. *Biochem. Biophys. Res. Commun.* **318**, 739–745
22. Elwell, C.A. and Dreyfus, L.A. (2000) DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest. *Mol. Microbiol.* **37**, 952–963
23. Elwell, C., Chao, K., Patel, K., and Dreyfus, L. (2001) *Escherichia coli* CdtB mediates cytolethal distending toxin cell cycle arrest. *Infect. Immun.* **69**, 3418–3422
24. Dlakic, M. (2001) Is CdtB a nuclease or a phosphatase? *Science* **291**, 547
25. Lara-Tejero, M. and Galan, J.E. (2000) A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. *Science* **290**, 354–357
26. Li, L., Sharipo, A., Chaves-Olarte, E., Masucci, M.G., Levitsky, V., Thelestam, M., and Frisan, T. (2002) The *Haemophilus ducreyi* cytolethal distending toxin activates sensors of DNA damage and repair complexes in proliferating and non-proliferating cells. *Cell. Microbiol.* **4**, 87–99
27. Hassane, D.C., Lee, R.B., and Pickett, C.L. (2003) *Campylobacter jejuni* cytolethal distending toxin promotes DNA repair responses in normal human cells. *Infect. Immun.* **71**, 541–545
28. Comayras, C., Tasca, C., Peres, S.Y., Ducommun, B., Oswald, E., and De Rycke, J. (1997) *Escherichia coli* cytolethal distending toxin blocks the HeLa cell cycle at the G2/M transition by preventing cdc2 protein kinase dephosphorylation and activation. *Infect. Immun.* **65**, 5088–5095
29. Whitehouse, C.A., Balbo, P.B., Pesci, E.C., Cottle, D.L., Mirabito, P.M., and Pickett, C.L. (1998) *Campylobacter jejuni* cytole-

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thal distending toxin causes a G2-phase cell cycle block. *Infect. Immun.* **66**, 1934–1940

30. Cortes-Bratti, X., Chaves-Olarte, E., Lagergård, T., and Thelestam, M. (1999) The cytolethal distending toxin from the chancroid bacterium *Haemophilus ducreyi* induces cell-cycle arrest in the G2 phase. *J. Clin. Invest.* **103**, 107–115
31. De Rycke, J., Sert, V., Comayras, C., and Tasca, C. (2000) Sequence of lethal events in HeLa cells exposed to the G2 blocking cytolethal distending toxin. *Eur. J. Cell Biol.* **79**, 192–201
32. Pickett, C.L. and Whitehouse, C.A. (1999) The cytolethal distending toxin family. *Trends Microbiol.* **7**, 292–297
33. Hassane, D.C., Lee, R.B., Mendenhall, M.D., and Pickett, C.L. (2001) Cytolethal distending toxin demonstrates genotoxic activity in a yeast model. *Infect. Immun.* **69**, 5752–5759
34. Escalas, N., Davezac, N., De Rycke, J., Baldin, V., Mazars, R., and Ducommun, B. (2000) Study of the cytolethal distending toxin-induced cell cycle arrest in HeLa cells: involvement of the CDC25 phosphatase. *Exp. Cell Res.* **257**, 206–212
35. Cortes-Bratti, X., Karlsson, C., Lagergård, T., Thelestam, M., and Frisan, T. (2001) The *Haemophilus ducreyi* cytolethal distending toxin induces cell cycle arrest and apoptosis via the DNA damage checkpoint pathways. *J. Biol. Chem.* **276**, 5296–52302
36. Sato, T., Koseki, T., Yamato, K., Saiki, K., Konishi, K., Yoshikawa, M., Ishikawa, I., and Nishihara, T. (2002) p53-independent expression of p21(CIP1/WAF1) in plasmacytic cells during G(2) cell cycle arrest induced by *Actinobacillus actinomycetemcomitans* cytolethal distending toxin. *Infect. Immun.* **70**, 528–534
37. Norbury, C.J. and Hickson, I. (2001) Cellular responses to DNA damage. *Annu. Rev. Pharmcol. Toxicol.* **41**, 367–401
38. Taylor, W.R. and Stark, G.R. (2001) Regulation of the G2/M transition by p53. *Oncogene* **20**, 1803–1815
39. Svensson, L.A., Henning, P., and Lagergard, T. (2002) The cytolethal distending toxin of *Haemophilus ducreyi* inhibits endothelial cell proliferation. *Infect. Immun.* **70**, 2665–2669
40. Gelfanova, V., Hansen, E.J., and Spinola, S.M. (1999) Cytolethal distending toxin of *Haemophilus ducreyi* induces apoptotic death of Jurkat T cells. *Infect. Immun.* **67**, 6394–6402
41. Shenker, B.J., Hoffmaster, R.H., Zekavat, A., Yamaguchi, N., Lally, E.T., and Demuth, D.R. (2001) Induction of apoptosis in human T cells by *Actinobacillus actinomycetemcomitans* cytolethal distending toxin is a consequence of G2 arrest of the cell cycle. *J. Immunol.* **167**, 435–441
42. Nalbant, A., Chen, C., Wang, Y., and Zadeh, H.H. (2003) Induction of T-cell apoptosis by *Actinobacillus actinomycetemcomitans* mutants with deletion of *ltxA* and *cdt*ABC genes: possible activity of GroEL-like molecule. *Oral Microbiol. Immunol.* **18**, 339–349
43. Ohara, M., Hayashi, T., Kusunoki, Y., Miyauchi, M., Takata, T., and Sugai, M. (2004) Caspase-2 and caspase-7 are involved in cytolethal distending toxin-induced apoptosis in Jurkat and MOLT-4 T-cell lines. *Infect. Immun.* **72**, 871–879
44. Shenker, B.J., Hoffmaster, R.H., McKay, T.L., and Demuth, D.R. (2000) Expression of the cytolethal distending toxin (Cdt) operon in *Actinobacillus actinomycetemcomitans*: evidence that the CdtB protein is responsible for G2 arrest of the cell cycle in human T cells. *J. Immunol.* **165**, 2612–2618
45. Belibasakis, G., Johansson, A., Wang, Y., Claesson, R., Chen, C., Asikainen, S., and Kalfas, S. (2002) Inhibited proliferation of human periodontal ligament cells and gingival fibroblasts by *Actinobacillus actinomycetemcomitans*: involvement of the cytolethal distending toxin. *Eur. J. Oral Sci.* **110**, 366–373
46. Toth, I., Oswald, E., Mainil, J.G., Awad-Masalmeh, M., and Nagy, B. (2000) Characterization of intestinal *cnf1*+ *Escherichia coli* from weaned pigs. *Int. J. Med. Microbiol.* **290**, 539–542
47. Morabito, S., Dell'Omo, G., Agrimi, U., Schmidt, H., Karch, H., Cheasty, T., and Caprioli, A. (2001) Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. *Vet. Microbiol.* **82**, 275–283
48. Marques, L.R., Tavechio, A.T., Abe, C.M., and Gomes, T.A. (2003) Search for cytolethal distending toxin production among fecal *Escherichia coli* isolates from Brazilian children with diarrhea and without diarrhea. *J. Clin. Microbiol.* **41**, 2206–2208
49. Van Bost, S., Roels, S., Oswald, E., and Mainil, J. (2003) Putative roles of the CNF2 and CDTIII toxins in experimental infections with necrotoxigenic *Escherichia coli* type 2 (NTEC2) strains in calves. *Microbes Infect.* **5**, 1189–1193
50. Mbwana, J., Ahmed, H.J., Ahlman, K., Sundaeus, V., Dahlen, G., Lyamuya, E., and Lagergard, T. (2003) Specificity of antibodies directed against the cytolethal distending toxin of *Haemophilus ducreyi* in patients with chancroid. *Microb. Pathog.* **35**, 133–137
51. Henderson, B., Nair, S.P., Ward, J.M., and Wilson, M. (2003) Molecular pathogenicity of the oral opportunistic pathogen *Actinobacillus actinomycetemcomitans*. *Annu. Rev. Microbiol.* **57**, 29–55
52. Kaplan, J.B., Schreiner, H.C., Furgang, D., and Fine, D.H. (2002) Population structure and genetic diversity of *Actinobacillus actinomycetemcomitans* strains isolated from localized juvenile periodontitis patients. *J. Clin. Microbiol.* **40**, 1181–1187
53. Ahmed, H.J., Svensson, L.A., Cope, L.D., Latimer, J.L., Hansen, E.J., Ahlman, K., Bayat-Turk, J., Klamer, D., and Lagergard, T. (2001) Prevalence of *cdt*ABC genes encoding cytolethal distending toxin among *Haemophilus ducreyi* and *Actinobacillus actinomycetemcomitans* strains. *J. Med. Microbiol.* **50**, 860–864
54. Fabris, A.S., DiRienzo, J.M., Wikstrom, M., and Mayer, M.P. (2002) Detection of cytolethal distending toxin activity and *cdt* genes in *Actinobacillus actinomycetemcomitans* isolates from geographically diverse populations. *Oral Microbiol. Immunol.* **17**, 231–238
55. Tan, K.S., Song, K.P., and Ong, G. (2002) Cytolethal distending toxin of *Actinobacillus actinomycetemcomitans*. Occurrence and association with periodontal disease. *J. Periodont. Res.* **37**, 268–272
56. Yamano, R., Ohara, M., Nishikubo, S., Fujiwara, T., Kawamoto, T., Ueno, Y., Komatsuzawa, H., Okuda, K., Kurihara, H., Suginaka, H., *et al.* (2003) Prevalence of cytolethal distending toxin production in periodontopathogenic bacteria. *J. Clin. Microbiol.* **41**, 1391–1398
57. Lee, R.B., Hassane, D.C., Cottle, D.L., and Pickett, C.L. (2003) Interactions of *Campylobacter jejuni* cytolethal distending toxin subunits CdtA and CdtC with HeLa cells. *Infect. Immun.* **71**, 4883–4890
58. Deng, K., and Hansen, E.J. (2003) A CdtA-CdtC complex can block killing of HeLa cells by *Haemophilus ducreyi* cytolethal distending toxin. *Infect. Immun.* **71**, 6633–6640
59. Cortes-Bratti, X., Chaves-Olarte, E., Lagergard, T., and Thelestam, M. (2000) Cellular internalization of cytolethal distending toxin from *Haemophilus ducreyi*. *Infect. Immun.* **68**, 6903–6911
60. Nishikubo, S., Ohara, M., Ueno, Y., Ikura, M., Kurihara, H., Komatsuzawa, H., Oswald, E., and Sugai, M. (2003) An N-terminal segment of the active component of the bacterial genotoxin cytolethal distending toxin B (CDTB) directs CDTB into the nucleus. *J. Biol. Chem.* **278**, 50671–55081
61. McSweeney, L.A. and Dreyfus, L.A. (2004) Nuclear localization of the *Escherichia coli* cytolethal distending toxin CdtB subunit. *Cell. Microbiol.* **6**, 447–458
62. Nesic, D., Hsu, Y., and Stebbins, C.E. (2004) Assembly and function of a bacterial genotoxin. *Nature* **429**, 429–433
63. De Rycke, J. and Ducommun, B. (2003) Bacterial cyclostatin, or how do bacteria manipulate the eukaryotic cell cycle. *Med. Sci. (Paris)* **19**, 1128–1136
64. Coburn, J. and Leong, J.M. (2000) Microbiology. Arresting features of bacterial toxins. *Science* **290**, 287–288
65. Lara-Tejero, M. (2001) A bacterial toxin that causes DNA damage to modulate cellular responses. *Sci. World J.* **1**, 190–191

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