Bacterial Toxins: Friends or Foes?

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Many emerging and reemerging bacterial pathogens synthesize toxins that serve as primary virulence factors. We highlight seven bacterial toxins produced by well-established or newly emergent pathogenic microbes. These toxins, which affect eukaryotic cells by a variety of means, include *Staphylococcus aureus* α-toxin, Shiga toxin, cytotoxic necrotizing factor type 1, *Escherichia coli* heat-stable toxin, botulinum and tetanus neurotoxins, and *S. aureus* toxic-shock syndrome toxin. For each, we discuss the information available on its synthesis and structure, mode of action, and contribution to virulence. We also review the role certain toxins have played in unraveling signal pathways in eukaryotic cells and summarize the beneficial uses of toxins and toxoids. Our intent is to illustrate the importance of the analysis of bacterial toxins to both basic and applied sciences.

Since diphtheria toxin was isolated by Roux and Yersin in 1888 (1), microbial toxins have been recognized as the primary virulence factor(s) for a variety of pathogenic bacteria. Bacterial toxins have been defined as "soluble substances that alter the normal metabolism of host cells with deleterious effects on the host" (2). Indeed, the major symptoms associated with disease caused by *Corynebacterium diphtheriae* (diphtheria), *Bordetella pertussis* (whooping cough), *Vibrio cholerae* (cholera), *Bacillus anthracis* (anthrax), *Clostridium botulinum* (botulism), *Clostridium tetani* (tetanus), and enterohemorrhagic *Escherichia coli* (bloody diarrhea and hemolytic uremic syndrome) are all related to the activities of the toxins produced by these organisms. With the recognition of the central role of toxin in these and other diseases has come the application of inactive toxins (toxoids) as vaccines. Such toxoid vaccines have had an important positive impact on public health.

In this review, we provide a summary overview (Table) of a variety of bacterial toxins categorized according to mode of action: damaging cell membranes, inhibiting protein synthesis, activating second messenger pathways, inhibiting the release of neurotransmitters, or activating the host immune response. We also describe in detail seven toxins: *Staphylococcus aureus* α-toxin, Shiga toxin (Stx), cytotoxic necrotizing factor type 1 (CNF1), *E. coli* heat-stable toxin (ST), botulinum and tetanus neurotoxins, and toxic-shock syndrome toxin (TSST) produced by *S. aureus*. We emphasize these toxins because they are produced by emerging (Stx of enterohemorrhagic *E. coli*) or reemerging (α-toxin of multidrug-resistant *S. aureus*) pathogens or illustrate different structures or modes of action (ST, CNF1, neurotoxins, and TSST).

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When It Rains, It Pores

Many bacterial exotoxins have the capacity to damage the extracellular matrix or the plasma membrane of eukaryotic cells. The damage not only may result in the direct lysis of cells but also can facilitate bacterial spread through tissues. Toxins that mediate this cellular damage do so by either enzymatic hydrolysis or pore formation. Bacterial hyaluronidases, collagenases, and phospholipases have the capacity to degrade cellular membranes or matrices. Specific examples of these types of toxins include the α-toxin of *Clostridium perfringens,* which has phospholipase C activity; *Streptococcus pyogenes* streptokinase, which can hydrolyze plasminogen to plasmin and dissolve clots; and the clostridial collagenases (3-5). Pore-forming toxins, as the

Table. Characteristics of bacterial toxins[a]

Organism/toxin	Mode of action	Target	Disease	Toxin implicated in disease[b]
Damage membranes				
Aeromonas hydrophila/aerolysin	Pore-former	Glycophorin	Diarrhea	(yes)
Clostridium perfringens/ perfringolysin O	Pore-former	Cholesterol	Gas gangrene[c]	?
Escherichia coli/hemolysin[d]	Pore-former	Plasma membrane	UTIs	(yes)
Listeria monocytogenes/ listeriolysin O	Pore-former	Cholesterol	Foodborne systemic illness, meningitis	(yes)
Staphyloccocus aureus/α-toxin	Pore-former	Plasma membrane	Abcesses[c]	(yes)
Streptococcus pneumoniae/ pneumolysin	Pore-former	Cholesterol	Pneumonia[c]	(yes)
Streptococcus pyogenes/ streptolysin O	Pore-former	Cholesterol	Strep throat, Sf[c]	?
Inhibit protein synthesis				
Corynebacterium diphtheriae/ diphtheria toxin	ADP-ribosyltransferase	Elongation factor 2	Diphtheria	yes
E. coli/*Shigella dysenteriae*/ Shiga toxins	N-glycosidase	28S rRNA	HC and HUS	yes
Pseudomonas aeruginosa/ exotoxin A	ADP-ribosyltransferase	Elongation factor 2	Pneumonia[c]	(yes)
Activate second messenger pathways				
E.coli				
CNF	Deamidase	Rho G-proteins	UTIs	?
LT	ADP-ribosyltransferase	G-proteins	Diarrhea	yes
ST[d]	Stimulates guanylate cyclase	guanylate cyclase receptor	Diarrhea	yes
CLDT[d]	G2 block	Unknown	Diarrhea	(yes)
EAST	ST-like?	Unknown	Diarrhea	?
Bacillus anthracis/edema factor	Adenylate cyclase	ATP	Anthrax	yes
Bordetella pertussis/				
dermonecrotic toxin	Deamidase	Rho G-proteins	Rhinitis	(yes)
pertussis toxin	ADP-ribosyltransferase	G-protein(s)	Pertussis	yes
Clostridium botulinum/C2 toxin	ADP-ribosyltransferase	Monomeric G-actin	Botulism	?
C. botulinum/C3 toxin	ADP-ribosyltransferase	Rho G-protein	Botulism	?
Clostridium difficile/				
toxin A	Glucosyltransferase	Rho G-protein(s)	Diarrhea/PC	(yes)
toxin B	Glucosyltransferase	Rho G-protein(s)	Diarrhea/PC	?
Vibrio cholerae/cholera toxin	ADP-ribosyltransferase	G-protein(s)	Cholera	yes
Activate immune response				
S. aureus/				
enterotoxins	Superantigen	TCR and MHC II	Food poisoning[c]	yes
exfoliative toxins	Superantigen (and serine protease?)	TCR and MHC II	SSS[c]	yes
toxic-shock toxin	Superantigen	TCR and MHC II	TSS[c]	yes
S. pyogenes/pyrogenic exotoxins	Superantigens	TCR and MHC II	SF/TSS[c]	yes
Protease				
B. anthracis/lethal factor	Metalloprotease	MAPKK1/MAPKK2	Anthrax	yes
C. botulinum/neurotoxins A-G	Zinc-metalloprotease	VAMP/synaptobrevin, SNAP-25, syntaxin	Botulism	yes
Clostridium tetani/tetanus toxin	Zinc-metalloprotease	VAMP/synaptobrevin	Tetanus	yes

[a]Abbreviations: CNF, cytotoxic necrotizing factor; LT, heat-labile toxin; ST, heat-stable toxin; CLDT, cytolethal distending toxin; EAST, enteroaggregative *E. coli* heat-stable toxin; TCR, T-cell receptor; MHC II, major histocompatibility complex class II; MAPKK, mitogen-activated protein kinase kinase; VAMP, vesicle-associated membrane protein; SNAP-25, synaptosomal-associated protein; UTI, urinary tract infection; HC, hemorrhagic colitis; HUS, hemolytic uremic syndrome; PC, antibiotic-associated pseudomembranous colitis; SSS, scalded skin syndrome; SF, scarlet fever; TSS, toxic-shock syndrome.
[b]Yes, strong causal relationship between toxin and disease; (yes), role in pathogenesis has been shown in animal model or appropriate cell culture; ?, unknown.
[c]Other diseases are also associated with the organism.
[d]Toxin is also produced by other genera of bacteria.

name suggests, disrupt the selective influx and efflux of ions across the plasma membrane by inserting a transmembrane pore. This group of toxins includes the RTX (repeats in toxin) toxins from gram-negative bacteria, streptolysin O produced by *S. pyogenes*, and the *S. aureus* α-toxin (described below).

S. aureus α-toxin can be considered the prototype of oligomerizing pore-forming cytotoxins. The α-toxin gene resides as a single copy on the chromosome of most pathogenic *S. aureus* strains, and its expression is environmentally regulated at the transcriptional level by the staphylococcal accessory gene regulator (*agr*) locus (6,7). The α-toxin is synthesized as a 319 amino acid precursor molecule that contains an N-terminal signal sequence of 26 amino acids. The secreted mature toxin, or protomer, is a hydrophilic molecule that lacks cysteine residues and has a molecular mass of approximately 33 kDa (6-8). Recently, the crystallographic structure of the fully assembled α-toxin pore was solved (9). On the plasma membrane, seven toxin protomers assemble to form a 232-kDa mushroom-shaped heptamer comprising three distinct domains (Figure 1A) (9,10). The cap and rim

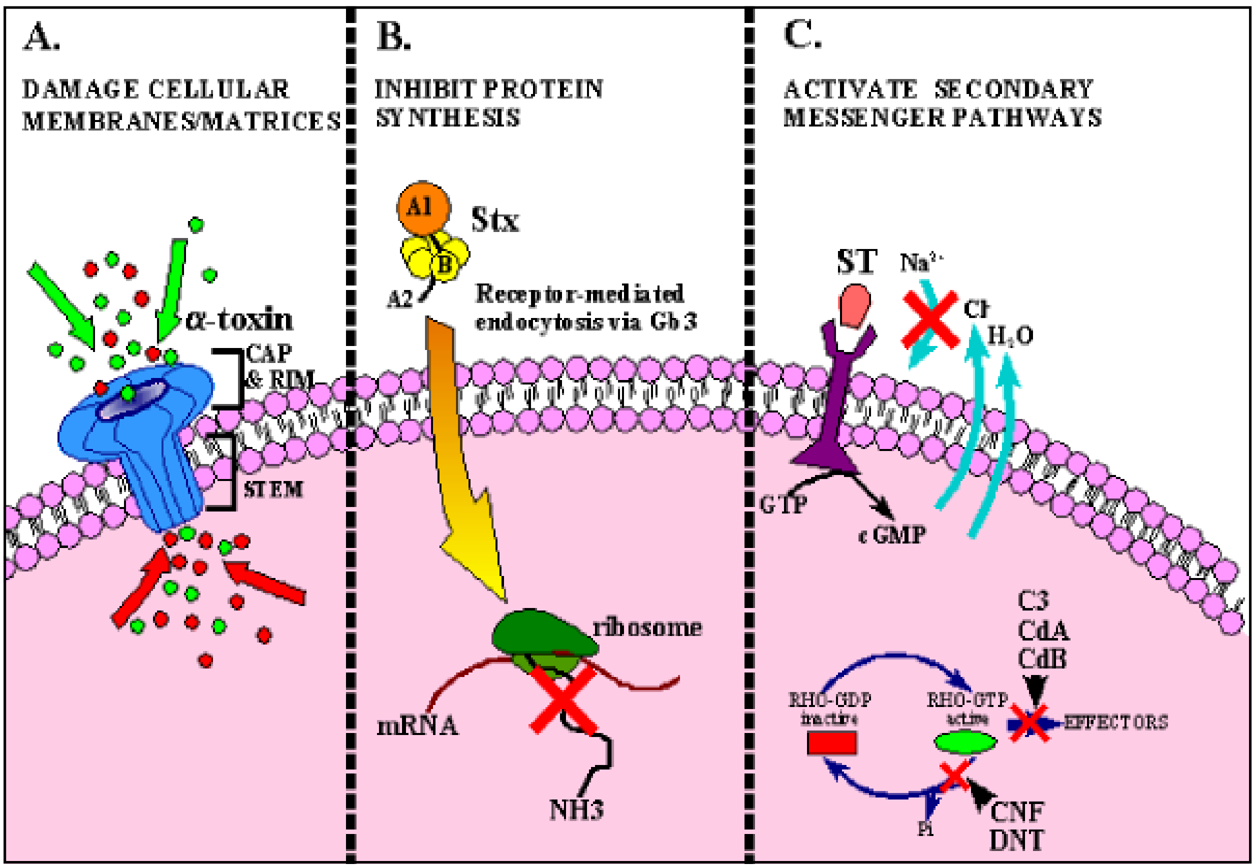

Figure 1. Diagrammatic representation of the mode of action of several bacterial toxins. A. Damage to cellular membranes by *Staphylococcus aureus* α-toxin. After binding and oligomerization, the stem of the mushroom-shaped α-toxin heptamer inserts into the target cell and disrupts membrane permeability as depicted by the influx and efflux of ions represented by red and green circles. B. Inhibition of protein synthesis by Shiga toxins (Stx). Holotoxin, which consists of an enzymatically active (A) subunit and five binding (B) subunits, enters cells through the globotriasylceramide (Gb3) receptor. The N-glycosidase activity of the A subunit then cleaves an adenosine residue from 28S ribosomal RNA, which halts protein synthesis. C. Examples of bacterial toxins that activate secondary messenger pathways. Binding of the heat-stable enterotoxins (ST) to a guanylate cyclase receptor results in an increase in cyclic GMP (cGMP) that adversely effects electrolyte flux. By ADP-ribosylation or glucosylation respectively, the C3 exoenzyme (C3) of *Clostridium botulinum* and the *Clostridium difficile* toxins A and B (CdA & CdB) inactivate the small Rho GTP-binding proteins**.** Cytotoxic necrotizing factor (CNF) of *E. coli* and the dermonecrotic toxin (DNT) of *Bordetella* species activate Rho by deamidation.

domains of the α-toxin heptamer are situated at the surface of the plasma membrane, while the stem domain serves as the transmembrane channel.

Alpha-toxin is cytolytic to a variety of cell types, including human monocytes, lymphocytes, erythrocytes, platelets, and endothelial cells (6,8). For α-toxin to damage cellular membranes, three sequential events are required. Toxin protomers must first bind to target membranes by either unidentified high-affinity receptors or through nonspecific absorption to substances such as phosphotidylcholine or cholesterol on the lipid bilayer (6-8). Second, membrane-bound protomers must oligomerize into a nonlytic prepore heptamer complex. Third, the heptamer must undergo a series of conformational changes that create the stem domain of the toxin, which is then inserted into the membrane (9,10). The α-toxin pore allows the influx and efflux of small molecules and ions that eventually lead to the swelling and death of nucleated cells and the osmotic lysis of erythrocytes. Pore formation has also been shown to trigger secondary events that could promote development of pathologic sequelae. These events include endonuclease activation, increased platelet exocytosis, release of cytokines and inflammatory mediators, and production of eicosanoids (6,8). Several animal models have demonstrated that α-toxin is required for *S. aureus* virulence in these systems (6,8); however, the precise role of α-toxin in staphylococcal diseases in humans remains unclear.

Stop, in the Name of Toxin

A second class of toxins intoxicates target cells by inhibiting protein synthesis. Substrates for toxins in this group are elongation factors and ribosomal RNA. Diphtheria toxin and *Pseudomonas* exotoxin A act by ADP-ribosylating elongation factor 2 (EF2) (11,12). The modified EF2 is no longer able to function in protein synthesis. Stxs, also called verotoxins, are produced by *Shigella dysenteriae* serotype 1 and the emerging pathogens designated Stx-producing *E. coli* (STEC). Stxs inactivate ribosomal RNA (by a mechanism described below) so that the affected ribosome can no longer interact with elongation factors (13,14). The inhibition of protein synthesis by this group of toxins ultimately results in death of the target cell.

Stxs are potent cytotoxins that can be divided into two antigenically distinct groups that share 50% to 60% homology: Stx/Stx1 and Stx2 (15-17). Stx and Stx1 are elaborated by *S. dysenteriae* serotype 1 and *E. coli*, respectively, and differ at only one amino acid. Stx2-type toxins have been found only in *E. coli* isolates and are quite diverse. While Stx2 is considered the prototype of this group, variants have been found that differ antigenically, in receptor specificity and in activation by intestinal mucus. Some of these attributes are the result of only one or two nucleotide differences in the toxin genes.

The *stx* of *S. dysenteriae* is invariably chromosomally located. The genes that encode Stx1 and Stx2 are carried chromosomally or by lysogenic bacteriophages. The genes that code for the A and B subunits of Stxs, *stxA* and *stxB*, are organized within an operon. The operator region of Stx/Stx1 (but not Stx2) contains a consensus fur box that is responsible for the iron-regulation of Stx and Stx1 production. Neither iron nor any other environmental factors examined affect the expression of Stx2. However, intestinal mucus enhances the activity of some Stx2 variants (18). The Stxs, which carry typical N-terminal leader sequences, are not actively secreted from the bacterial cell and are thought to be released into the milieu during cell lysis.

Stxs display an AB-toxin structure; an enzymatically active A subunit is noncovalently associated with a binding, or B, component. The crystal structures of the Stx1 B pentamer (19) and the Stx holotoxin have been solved (20) (Figure 2). Other toxins that share this AB structure are the *E. coli* heat-labile toxin (21), cholera toxin, and pertussis toxin (22) (Figure 2). The molecular masses of mature Stx A and B monomeric subunits are approximately 35 kDa and 7.5 kDa, respectively, although holotoxin contains five B subunit molecules. The B subunit pentamer directs the binding of the holotoxin to sensitive eukaryotic cells via specific glycolipid receptors. Once internalized, the A polypeptide is cleaved into an enzymatically active A_1 portion and an A_2 portion; these fragments remain associated through a disulfide bond. The A_2 portion serves to link the A_1 fragment and the B pentamer.

The enzymatic A subunit acts as a specific *N*-glycosidase to cleave a single adenine residue from 28S ribosomal RNA (13,14). This

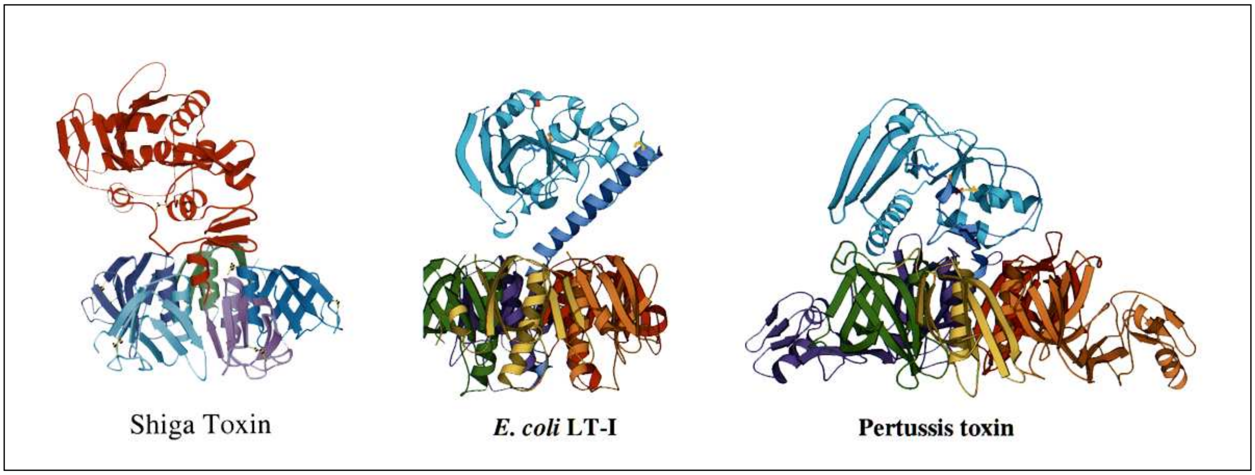

Figure 2. Ribbon crystal structures of *Shigella dysenteriae* Shiga toxin (20), *Escherichia coli* heat-labile toxin I (LT-I) (21), and pertussis toxin (22). The Shiga toxin figure was contributed by Marie Frasier. The LT-I and pertussis figures were contributed by Ethan Merritt. The figures were drawn in MOLSCRIPT (75).

depurination ultimately results in the inhibition of protein synthesis within intoxicated cells (Figure 1B). Prokaryotic ribosomes are as sensitive to the *N*-glycosidase activity of Stx as eukaryotic ribosomes (23).

STEC are considered emerging pathogens (24) because they were first described less than 20 years ago, during a 1983 outbreak of hemorrhagic colitis associated with undercooked hamburger (25,26). STEC O157:H7 causes approximately 20,000 cases of hemorrhagic colitis each year in the United States (27). Approximately 1,000 cases of the life-threatening sequelae hemolytic uremic syndrome and approximately 100 deaths are also attributed to *E. coli* O157:H7 annually in the United States (27).

Don't Shoot the Messenger

Bacterial toxins can also target and alter the function of a variety of cellular proteins without directly killing the intoxicated cell. Toxin activation or modification of secondary messengers can cause dramatic alterations to signal transduction pathways critical in maintaining a variety of cellular functions. To demonstrate the diversity among the toxins that belong to this category, we will describe CNF type 1 and the heat-stable enterotoxins.

Cytotoxic Necrotizing Factor (CNF)

CNF types 1 and 2 (CNF1/2) from *E. coli* belong to a group of bacterial toxins that modify Rho, a subfamily of small GTP-binding proteins that are regulators of the actin cytoskeleton (28,29). Most members of this toxin family, which includes the large clostridial cytotoxins and the C3 exoenzyme of *C. botulinum*, inactivate Rho (29). CNF1, CNF2, and the dermonecrotic toxins from *Bordetella* species form a unique subset in this family, since these toxins have the capacity to activate Rho (Figure 1C) (29-32). CNF1 and CNF2 share 99% amino acid similarity; however, we will discuss only CNF1 in detail because of its association with extraintestinal *E. coli* infections in humans, most notably urinary tract infections.

The gene for CNF1 is chromosomally encoded and resides on a pathogenicity island in uropathogenic *E. coli* (33,34). The toxin is synthesized as a hydrophilic polypeptide of approximately 115 kDa that remains primarily cytoplasmic because of the lack of a signal sequence (33). Recent structure and function analysis of CNF1 indicates that the toxin has distinct binding and enyzmatic domains (35). The N-terminal half of CNF1, which includes two potential transmembrane domains, contains the cellular binding domain. This region of the molecule shows amino acid similarity to the *Pasteurella multicoda* toxin, a potent mitogen thought to be the etiologic agent of progressive atrophic rhinitis in pigs (33,35). The C-terminal portion of CNF1 represents the toxin's enzymatic domain and shows homology with dermonecrotic toxins in a 100-amino acid stretch that may represent the active site of the toxin (33,35).

Eukaryotic cells intoxicated with CNF1 exhibit membrane ruffling; the formation of focal adhesions and actin stress fibers; and DNA replication in the absence of cell division, a phenomenon that results in enlarged multinucleated cells (Figure 3). The drastic changes apparent in CNF1-treated cells are a result of the toxin's capacity to modify Rho (29,30,32). This modification has recently been identified as a deamidation of the glutamine residue at position 63 of Rho to a glutamic acid. This amino acid change produces a dominant active Rho protein unable to hydrolyze bound GTP (30,32). In vivo, CNF1 causes necrosis in rabbit skin following intradermal injection and persistent inflammation in a mouse footpad assay (36). Epidemiologic data support the role of CNF1 as a virulence factor in human extraintestinal infections, although direct proof of the toxin's role in disease remains to be determined (29,37).

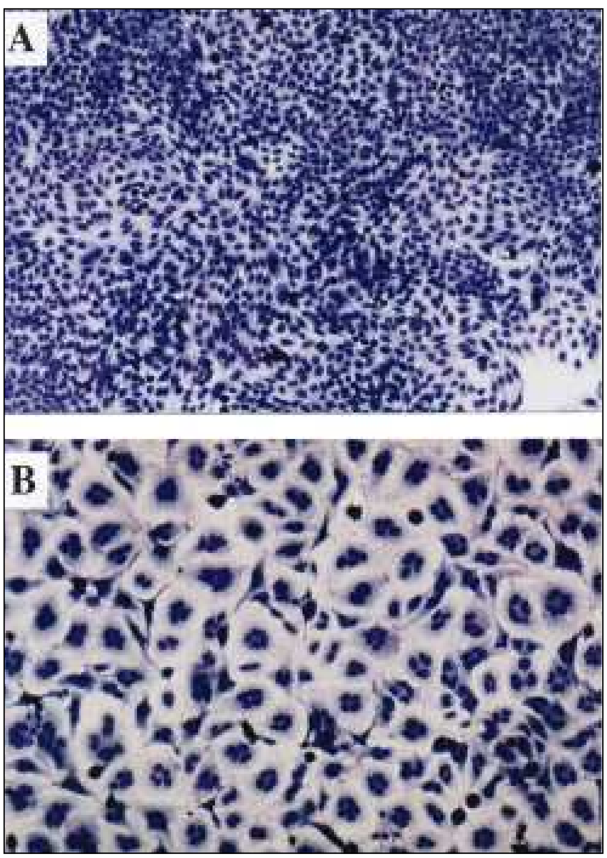

Figure 3. The effect of cytotoxic necrotizing factor type 1 (CNF1) on eukaryotic cells. A. HEp-2 cells, magnification 10X. B. HEp-2 cells intoxicated with CNF1, magnification 10X.

Heat-Stable Toxin (ST)

Two families of diarrheagenic STs have been described: STa (or STI) and STb (or STII). Distinct STas are produced by a variety of enteric pathogenic organisms: enterotoxinogenic *E. coli* (ETEC) (the focus of this section), *V. cholerae*, *Vibrio mimicus*, *Yersinia enterocolitica*, *Citrobacter freundii*, and *Klebsiella*.

Strains of ETEC associated with human disease may produce either STa, heat-labile toxin I, or both. STas from ETEC isolates are related but distinct toxins (38). STh is produced by strains of human origin, while STp is found predominantly in porcine strains. The STa genes (*estA*) of ETEC are encoded within a transposable element and have been found on a variety of replicons (39,40). STa is translated as a precursor molecule of 72 amino acids and undergoes two cleavage events before the secretion of the mature form into the culture supernatant. Mature STs are small peptides that range from 17 to 53 amino acids. STh and STp contain 19 and 18 residues, respectively. STas share a conserved C-terminal region of 13 amino acids essential for toxicity and the heat-stable nature of the toxin. Six cysteine residues are present within this domain, and the three disulphide bonds formed between the cysteine residues are necessary for toxicity of the molecule. Binding of STa to its cellular receptor results in the stimulation of membrane-bound guanylate cyclase, which in turn leads to an increase in intracellular cyclic GMP (Figure 1C) (41). This increase in cyclic GMP affects electrolyte flux in the bowel; sodium absorption is inhibited and chloride secretion is stimulated. These ion flux changes result in the secretory diarrhea characteristic of ETEC infection. ETEC cause traveler's diarrhea and are a major source of childhood diarrhea in many parts of the world.

The Nerve of Some Toxins

The *C. botulinum* neurotoxins (BoNTs, serotypes A-G) and the *C. tetani* tetanus neurotoxin (TeNT) constitute another category of bacterial toxins on the basis of similarities in structure, enzymatic activity, and the targeting to cells of the nervous system. BoNTs are most commonly associated with infant and foodborne botulism and exist in nature as large complexes comprised of the neurotoxin and one or more associated proteins believed to provide protection and stability to the toxin molecule while in the gut (42,43). TeNT, which is synthesized from vegetative *C. tetani* in wounds, does not appear to form complexes with any other protein components (42,43).

The BoNTs and TeNT are either plasmid encoded (TeNT, BoNTs/A, G, and possibly B) or bacteriophage encoded (BoNTs/C, D, E, F), and the neurotoxins are synthesized as inactive polypeptides of 150 kDa (44). BoNTs and TeNT are released from lysed bacterial cells and then activated by the proteolytic cleavage of an exposed loop in the neurotoxin polypeptide (45). Each active neurotoxin molecule consists of a heavy (100 kDa) and light chain (50 kDa) linked by a single interchain disulphide bond (42,45). The heavy chains of both the BoNTs and TeNT contain two domains: a region necessary for toxin translocation located in the N-terminal half of the molecule, and a cell-binding domain located within the C-terminus of the heavy chain (45,46). The light chains of both the BoNTs and TeNT contain zinc-binding motifs required for the zinc-dependent protease activities of the molecules (45,46).

The cellular targets of the BoNTs and TeNT are a group of proteins required for docking and fusion of synaptic vesicles to presynaptic plasma membranes and therefore essential for the release of neurotransmitters. The BoNTs bind to receptors on the presynaptic membrane of motor neurons associated with the peripheral nervous system. Proteolysis of target proteins in these neurons inhibits the release of acetylcholine, thereby preventing muscle contraction (47,48). BoNTs/B, D, F, and G cleave the vesicle-associated membrane protein and synaptobrevin, BoNT/A and E target the synaptosomal-associated protein SNAP-25, and BoNT/C hydrolyzes syntaxin and SNAP-25 (42,45,46). TeNT affects the central nervous system and does so by entering two types of neurons. TeNT initially binds to receptors on the presynaptic membrane of motor neurons but then migrates by retrograde vesicular transport to the spinal cord, where the neurotoxin can enter inhibitory interneurons (45,47). Cleavage of the vesicle-associated membrane protein and synaptobrevin in these neurons disrupts the release of glycine and gamma-amino-butyric acid, which, in turn, induces muscle contraction (47,48). The contrasting clinical manifestations of BoNT or TeNT intoxication (flaccid and spastic paralysis, respectively) are the direct result of the specific neurons affected and the type of neurotransmitters blocked (45-47).

Bacterial Superantigens: Too Much of a Good Thing

Several bacterial toxins can act directly on the T cells and antigen-presenting cells of the immune system. Impairment of the immunologic functions of these cells by toxin can lead to human disease. One large family of toxins in this category are the pyrogenic toxin superantigens (PTSAgs), whose hallmark biological activities include potent stimulation of the immune cell system, pyrogenicity, and enhancement of endotoxin shock (49-51). These stable, secreted toxins of 22 kDa to 30 kDa include staphylococcal enterotoxins serotypes A-E, G, and H; group A streptococcal pyrogenic exotoxins serotypes A-C and F; group A streptococcal superantigen; and staphylococcal TSST-1, which we discuss below.

All PTSAgs share common biological activities, but TSST-1 is the most divergent member of the toxin family, with less than 30% amino acid homology to other family members (52-54). TSST-1 is chromosomally encoded, and the *tst* gene is located in a variable genetic element in *S. aureus* (49,52,55). The toxin is synthesized as a precursor molecule of 234 residues with the first 40 amino acids acting as a signal sequence that is cleaved to generate the mature 22 kDa toxin (49). Expression of TSST-1 depends on oxygen, temperature, pH and glucose levels, and is regulated by the *S. aureus agr* locus (49,51). On the basis of crystallographic analysis, TSST-1 appears structurally similar to several other PTSAgs in that the toxin consists of two distinct domains; however, unlike other family members, TSST-1 does not require a zinc cofactor (51-54). Domain A of TSST-1 (amino acid residues 1-17 and 90-194) exists as a ß-grasp motif, and domain B consists of a five-stranded ß-barrel motif that forms an oligosaccharide/oligonucleotide binding fold.

In general, the potent immunostimulatory properties of PTSAgs are a direct result of toxin binding to distinct regions outside the peptide binding cleft of the major histocompatibility class II molecules (expressed on the surface of antigen-presenting cells) and to specific Vß elements on the T-cell receptor. In particular, the domain B of TSST-1 binds primarily to the α-chain of human leukocyte antigen-DR1 molecules, while domain A specifically binds to human T-cell receptor Vß2 elements (51-53,56). Binding of TSST-1 to Vß2 T-cell receptor elements results in a massive proliferation of up to 20% of peripheral T cells, an event that drastically skews the T-cell Vß repertoire (53,56). T cells that undergo this expansion can subsequently exist in a state of anergy or undergo apoptosis (56). Concomitant to T-cell proliferation is a massive release of both lymphocyte (interleukin [IL]-2, tumor necrosis factor ß, gamma interferon)-derived and monocyte (IL-1, IL-6, tumor necrosis factor α)-derived cytokines (51,56). These cytokines serve as mediators of the hypotension, high fever, and diffuse erythematous rash that are characteristic of toxic-shock syndrome. Long established as a key substance in causing staphylococcal toxic-shock syndrome, TSST-1 has more recently been linked with Kawasaki syndrome, a leading cause of acquired heart disease in children in the United States (50,54).

Dr. Jekyll or Mr. Hyde?

Some of these powerful disease-causing toxins have been exploited to further basic knowledge of cell biology or for medical purposes. For example, cholera toxin and the related labile-toxin of *E. coli*, as well as *B. pertussis* toxin, have been used as biologic tools to understand the mechanism of adenylate cyclase activation and the role of cyclic AMP as a second messenger in the eukaryotic cell (57-59). Derivatives of some of these toxins, cholera toxin and labile toxin, have also been incorporated into human vaccines because of the adjuvant properties of these molecules (60,61).

Similarly, the activities of several potent cytotoxins have been harnessed as potential therapies for certain cancers. Such toxins can either be used directly in treatment or as components of immunotoxins (62-64). For example, Stx binds to the cell surface glycolipid CD77, which is expressed by B cells in certain B-cell lymphomas (65,66). This finding led to studies that showed that Stx can purge murine (and potentially human) bone marrow of malignant CD77$^+$ B cells before an autologous bone marrow transplant (67). Other toxins that inhibit protein synthesis, such as diphtheria toxin, *Pseudomonas* exotoxin A, or the plant toxin ricin, are frequently engineered as the cell-killing component of immunotoxins. These "magic bullets," hybrids of the enzymatically active portion of a toxin molecule and monoclonal antibodies (or a receptor), are in clinical trials for the treatment of persons with B-cell lymphomas, leukemia, and bone marrow transplants.

Several clinical applications have also been found for the powerful botulinum neurotoxin type A (BoNT/A) (46,68). The disorders that respond to BoNT/A involve muscle hyperactivity. A minuscule amount of purified toxin injected into specific sites results in paralysis of the target muscle and ablation of the muscle spasm. Therapy must be continual since the effect of the toxin usually lasts for no more than several months. The first maladies treated with BoNT/A were eye movement abnormalities (69). However, the therapeutic value of BoNT/A has been shown for many other disorders including cervical and laryngeal dystonia, writer's cramp, hemifacial spasm, tremors, and tics (46,68). BoNT/A is also used cosmetically to reduce deep wrinkles caused by the contraction of facial muscles (70).

Another toxic bacterial product with medical applications is streptokinase, a potent plasminogen activator produced by several pathogenic streptococcal strains. The proteolytic activity of streptokinase is used to clear blocked arteries in patients who have heart attacks (71,72).

Vaccinate, Don't Procrastinate

Vaccines directed at the toxic component of bacterial pathogens have proven quite effective in preventing certain diseases. Most licensed toxoid vaccines are relatively crude, but effective, preparations. These vaccines consist of partially purified toxin preparations obtained from culture supernatants of bacteria such as *C. diphtheriae*, *C. tetani*, or *B. anthracis*. Formaldehyde treatment is used to detoxify the diphtheria and tetanus toxins for vaccine formulation. The anthrax vaccine contains the protective antigen and small amounts of the lethal factor and edema factor toxins. The current botulinum vaccine is an investigational drug composed of crude preparations of five botulinum toxoids and is distributed by the Centers for Disease Control and Prevention to researchers that work with the toxin or organism. Acellular pertussis vaccines that contain pertussis toxoid, alone or as one of several components, are as effective as killed whole-cell vaccines but less reactogenic (73);

such vaccines have recently been approved for use in infants as well as older children.

New vaccines aimed at toxins are in various stages of development: research and development, preclinical, phase I, phase II, or phase III (74). The next generation of toxoid vaccines falls into three general categories: purified toxoids that have been inactivated by chemical or genetic means; live, attenuated strains of the causative agent that produce a genetically derived toxoid; or live, attenuated unrelated bacterial vector strains, such as *V. cholerae* or *Salmonella,* that produce the target toxoid. Examples of each of these approaches and progress in development of specific toxoid vaccines are described annually in the Jordan Report (74).

Antitoxins raised against diphtheria, tetanus, and botulinum toxoids have also been used for many years to treat seriously ill patients. Antiserum specific for the Stx toxins produced by *E. coli* O157:H7 and other STEC is under development for the treatment and prevention of hemolytic uremic syndrome, a life-threatening sequela of these infections.

Summary

Microbial toxins capable of interrupting or hyperstimulating many essential functions and pathways of eukaryotic cells have evolved along with the carrier bacterium. Presumably these toxins confer some benefit to the bacterium, either during a stage of the host-parasite interaction or in some environmental niche encountered by the bacterium. Certain bacterial toxins act on the target cell surface to irreparably damage the cell membrane or alter normal cellular signal transduction. Other toxins exhibit enzymatic activity once the molecule has gained access to the cytoplasm of the sensitive cell by endocytosis. Yet other bacterial toxins act by either turning off or locking on a normal host cell function.

Although detrimental to the susceptible host during an infection, the activities of several bacterial toxins have been exploited as probes of eukaryotic cellular pathways and for medicinal applications. Thus, research on a microbial toxin produced by an established, emerging, or reemerging pathogen is likely to yield novel information about the role of that toxin in disease as well as the properties of host cells that are subverted by the toxin.

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