Bacterial Toxins

MICHAEL M. LUBRAN, M.D., Ph.D.

Department of Pathology, Harbor-UCLA Medical Center, Torrance, CA 90509

ABSTRACT

Many bacterial toxins are proteins, encoded by the bacterial chromosomal genes, plasmids or phages. Lysogenic phages form part of the chromosome. The toxins are usually liberated from the organism by lysis, but some are shed with outer membrane proteins in outer membrane vesicles. An important non-protein toxin is lipopolysaccharide or endotoxin, which is a constituent of the cell wall of gram negative bacteria. Toxins may damage the eukaryotic cell membrane by combining with some structural component, or otherwise alter its function. Many toxins combine with specific receptors on the surface membrane, frequently glycoproteins or gangliosides, and penetrate the cell to reach their intracellular target. A common mechanism of entry is absorptive endocytosis. Many protein toxins have an A-B structure, B being a polypeptide which binds to the receptor and A being an enzyme. Many toxins are activated, either when produced by the bacterium or when bound to the membrane receptor, by proteases (nicking). An enzymatic process common to many toxins is adenosine diphosphate (ADP)-ribosylation of the adenylate cyclase regulatory proteins, leading to an increase in intracellular cyclic adenosine monophosphate (cAMP). This is the mechanism of action of cholera toxin. Diphtheria toxin catalyzes the transfer of ADP-ribose to elongation factor-2, inhibiting protein synthesis. Most toxins act on the target cells to which they bind, but tetanus toxin, and, to a lesser degree, botulinum toxin, ascend axons and affect more distant structures. Although many toxin effects caused by bacteria have been described, only a few toxins have been identified, characterized, and their mode of action determined at the molecular level. The best known of these are discussed.

Introduction

Toxins produced by pathogenic bacteria interfere with the physiological functions of cells and are sometimes lethal. They act on the cell membrane (e.g., hemolysins, lysins, phospholipases) or some intracellular target. Occasionally, they are the sole cause of disease; in most cases, they act in concert with other virulence factors which enable the bacteria to establish themselves in the host and resist or evade its defensive mechanisms. Although bacterial toxins
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have been studied for many years, only a few have been fully characterized and their mode of action determined at a molecular level. Many of the toxins are proteins produced within the organisms. Exotoxins are exported through the bacterial membranes or released by lysis of the organisms. Gram negative bacteria have an outer membrane containing a lipopolysaccharide endotoxin. This toxin escapes from the organism in vesicles shed from the outer membrane. Toxins are also classified by their target organs or cells, for example, neurotoxins, enterotoxins, and cytotoxins; however, one toxin may act on different target cells. The effects of toxins have been studied in vivo in intact animals and in vitro on cells or cell fractions, using whole bacteria, bacterial fractions or cell-free extracts. The sensitivity of different animals and cells to toxins varies considerably, and it is often uncertain which effects observed experimentally can be extrapolated to human disease.

Bacteria and their toxins are listed in tables I and II. Many protein toxins have similar or related properties, which will be discussed in general and in more detail for the different toxins: these properties are export, surface binding, internalization (or translocation), action on target structures and genetic control.

### TABLE II
**Principal Toxins Produced by Pathogenic Bacteria**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Mycosides, cord factor, sulfatides</td>
</tr>
<tr>
<td>Neisseria meningitides</td>
<td>Endotoxin</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Exotoxin A</td>
</tr>
<tr>
<td>Salmonella (strains)</td>
<td>Enterotoxin, cytoxin</td>
</tr>
<tr>
<td>Shigella</td>
<td>Cytotoxin (Shiga)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Enterotoxins, pyrogens, toxic shock toxin (TET), exfoliatin, leukocidins, α-, β-, γ-, δ-toxins</td>
</tr>
<tr>
<td>Streptococcus (group A, β-hemolytic)</td>
<td>Torulysins, erythrogenic toxin</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>Pneumolysin</td>
</tr>
<tr>
<td>V. cholera</td>
<td>Enterotoxin</td>
</tr>
<tr>
<td>V. mimicus</td>
<td>Enterotoxin</td>
</tr>
<tr>
<td>V. parahemolyticus</td>
<td>Kanagawa hemolysin</td>
</tr>
<tr>
<td>V. vulnificus</td>
<td>Enterotoxin</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>Enterotoxin</td>
</tr>
</tbody>
</table>

### Protein Toxin Export in Bacteria

The protein toxins are synthesized on ribosomes as single polypeptide chains with a signal aminoacid sequence comprising about ten hydrophobic residues at its NH₂ terminal. In gram positive organisms, which have a single membrane, the protein traverses the membrane by diffusion or by creating a pore. The signal peptide is removed by a protease synthesized concurrently with the toxin, acting at the membrane. In many
cases, further mild proteolysis occurs outside the organism (nicking), resulting in two polypeptide chains held together by one or more disulfide bonds.

Protein export is more complicated in gram negative bacteria. The cell envelope consists of three layers or compartments: an inner, cytoplasmic membrane, an outer membrane, and a periplasmic space between them. The outer membrane is about 75 Å thick and is a bilayer consisting of two leaflets, an outer one of lipopolysaccharide and an inner one made of phospholipids. The periplasmic space contains a peptidoglycan membrane which gives the organism its rigid shape. The outer membrane contains porous areas, filled with proteins (porins), which allow the passage of small molecules but not proteins. Some toxins are released by cell lysis; others pass into the lipopolysaccharide of the outer membrane and reach the exterior in outer membrane vesicles as a protein-lipopolysaccharide complex. True secretion of proteins by gram negative bacteria is unusual, although it may occur with some hemolysins.

The complexity of protein export is well illustrated by the excretion of heat-labile toxin (LT) by Escherichia coli. This toxin, as detailed, is composed of A and B protomers. Both LT-A and LT-B are synthesized with signal peptides by cytoplasmic membrane-bound ribosomes. The LT-B is elongated rapidly, processed by a signal peptidase and immediately assembled into B₅-pentamers and transferred to the periplasmic space. The LT-A is formed more slowly and remains associated externally with the cytoplasmic membrane until it meets and combines with a B₅-pentamer, forming the soluble toxin molecule, which is shed in outer membrane vesicles.

**Cell Surface Binding of Protein Toxins**

Most toxins have a high affinity for specific cells, although a few toxins, such as cholera toxin, can affect a wide range of cells in vitro. In human cholera, however, the toxin acts mainly as an enterotoxin. Specificity is related to the presence on the cell surface of membrane receptors specific for a particular toxin. The receptors may be glycoproteins, gangliosides, which may also act as receptors of neurotransmitters and other physiological substances, sterols, and other molecules of unknown composition. The receptors are usually randomly distributed on the surface membrane. In some cases, the ligand-receptor complexes of toxins acting intracellularly cluster after binding into specialized areas of the membrane termed "coated pits", which are lined with clathrin, a filamentous membrane protein. Clustering into coated pits is aided by a cross-linking enzyme, transglutaminase. This process results in the concentration of toxin molecules from a dilute solution and their localization to specific regions of the surface membrane.

Some protein toxins of high molecular weight are made up of two protomers, A and B. The active part of the toxin molecule, A, is responsible for its toxicity. It is usually an enzyme, acting on an intracellular target; B may be composed of smaller subunits. It is responsible for binding the toxin molecule to the receptor and may aid in releasing A from combination, so that it may enter the cell; B remains on the surface. Some toxins, such as cholera toxin, bind to specific receptors and are translocated as the complete toxin, not as subunits. The mode of binding of many toxins is not known.

**Translocation of Protein Toxins**

Small molecular weight toxins probably enter the cell by simple pinocytosis. A small amount of surrounding fluid containing the toxin is enclosed in a pino-
cystic vesicle and moves into the cytoplasm. This is an inefficient process, and its success depends on the presence of a high concentration of toxin in the neighborhood of the cell. High molecular weight toxins are translocated by absorptive endocytosis.

In this receptor-mediated process (RME), the toxin-receptor complexes undergo translational diffusion, are combined enzymatically, are concentrated in coated pits, as described previously, and pass into the cytoplasm as endosomes. The toxins leave the endosomes by different processes (which will be described later) and reach their intracellular target.

Surface-active toxins exert their effects on specific cells by binding to components of the surface membrane and destabilizing it. Hemolysins bind to cholesterol causing a leak of potassium ions and then hemolysis. Toxins acting on leukocytes (e.g. streptolysins O and S, Str. pneumoniae pneumolysin) combine with surface-membrane sterols or phospholipids resulting in release of lysosomal hydrolytic enzymes, degranulation and cell death. Lesser effects are suppression of chemotaxis and phagocytosis. Lysogenic streptococci (i.e., having prophage desoxyribonucleic acid [DNA] incorporated in the bacterial DNA) produce an erythrogenic toxin, which is also pyrogenic. It enhances susceptibility to endotoxic shock and cardiotoxicity. The β-toxin of Staph. aureus is an enzyme which reacts with sphingomyelin to split off phosphorylcholine. Leukocidins act by stimulating membrane acylphosphatase to dephosphorylate membrane triphosphoinositide.

Protein synthesis is affected by some toxins, for example, diphtheria toxin and exotoxin A produced by Pseudomonas aeruginosa. The A subunit of these toxins is activated by lysosomal enzymes in the perinuclear region. The free A subunits bind to a specific aminoacid residue, diphthamide (a histidine derivative), in elongation factor 2 (EF-2), inhibiting its action and disrupting protein synthesis and the cell dies.

Adenylate cyclase activity is increased by cholera toxin, EC-LT and pertussis toxin from Bordetella pertussis. The enzyme is bound to the cytoplasmic side of the surface membrane. Adenylate cyclase consists of a hormone receptor, a catalytic portion and two guanylic nucleotide-binding proteins, G_s and G_i, which, respectively, regulate stimulation and inhibition of the enzyme by surface-bound hormones. Cholera toxin and EC-LT ADP-ribosylate the α-subunit of G_s; pertussis toxin ADP-ribosylates the α-subunit of G_i. These are two different proteins, with respective Mr's of 45,000 and 41,000. The β-subunits of these proteins are the same. The A_1 subunit of the cholera toxin and EC-LT cleaves NAD^+ and transfers the resulting ADP-ribose to the α-subunit of G_s. The ADP-ribosylated α-subunit has less affinity for the β-subunit (of G_i), which increases in concentration and stimulates cAMP production. Pertussis toxin ADP-ribosylates the inhibitory regulator protein G_i and allows the stimulatory regulator to increase cAMP concentration. An unregulated increase in cAMP concentration is equivalent to continued overstimulation of the cell by hormones such as noradrenaline and eventually leads to the death of the cell.

Genetic Control of Protein Toxin Production

Genes controlling the production of protein toxins may reside in the bacterial chromosome, phage particles or plasmids. The DNA of temperate phages is incorporated into the bacterial chromosome. Phage reproduction is turned off by repression of early and late gene functions: the repressed phage genome remains in the cell as prophage and replicates synchronously with the cell (lysogeny). Occasionally, repression of the
prophage fails and the cells are lysed. Lysis can also be induced by external factors affecting DNA replication. Plasmids and phages may be transferred from a toxin-producing strain to a non-toxin-producing strain, making the bacteria toxin-producing. Genetic control is discussed with the individual toxins.

Diphtheria Toxin

Diphtheria toxin is a single polypeptide of Mr 58,342 secreted by toxic strains of Corynebacterium diphtheriae, carrying the tox structural gene found in the lysogenic corynebacteriophages $\beta$ tox$^+$, $\gamma$ tox$^+$ and $\omega$ tox$^+$. Avirulent strains become lysogenic when infected with these phages. Highly toxic strains have two or three tox$^+$ genes inserted into the genome. Expression of this gene is regulated by the bacterial host. The repressor gene is iron-dependent: its action is inhibited, and toxin production is enhanced in the presence of very low concentrations of iron. The toxin is synthesized as a single polypeptide chain of 535 residues, with a 25 residue leader sequence and two disulfide bridges. The signal peptide is cleaved by a concomitantly produced protease as the toxin is shed in the outer membrane vesicles. After export from the organism, the protein is cleaved by a proteolytic enzyme into two subunits, A and B, held together by an interchain disulfide bond between cystine residues at 186 and 201. Reduction of the disulfide bond causes separation of the A and B subunits, but they remain closely associated by non-covalent forces. The A subunit (which has 193 residues and Mr, 21,150) is an enzyme, which catalyzes the ADP-ribosylation of elongation factor-2, which is involved in the translocation process of protein synthesis. The B subunit is responsible for binding the intact toxin molecule to specific receptors in the surface membrane of the target cells. The B chain has two $\alpha$-helical amphipathic domains, reminiscent of the domains of apolipoproteins, which react with the membrane phospholipids and core lipids; there is a third hydrophobic domain which anchors the B chain through the thickness of the membrane. The nature of the surface membrane receptor is not known. Glycoproteins of Mr, 150,000 have been isolated which can bind diphtheria toxin in vitro, but their physiological role has not been established. The receptor area probably occupies a broad region of the surface and may consist of several functional groups.

Internalization of the toxin occurs mainly by receptor-mediated endocytosis. Some toxin molecules may enter the cell by direct plasma membrane transversal through non-specific pinocytosis, but very few molecules survive in the cytoplasm to reach their target. In RME, the toxin-receptor complexes gather into coated pits lined with clathrin. The pits form vesicles which migrate towards the Golgi apparatus and fuse with primary lysosomes. The B chain, because of its hydrophobic domains, becomes embedded in the lipid membrane of the endosomes and creates a pore. The lysosomal proteases cleave the toxin molecule into its A and B chains, which become separated by reduction of the disulfide bridge. The acid milieu produced by the lysosomes causes a conformational change in the A chain, which can leave the endosome through the pore and pass to its target. The A chain is very stable and is not attacked by the lysosomal enzymes; the B chain, however, is readily destroyed.

Interaction of the A chain with EF-2 involves binding to NAD$^+$ at a single site near glu-148. The complex then binds to EF-2 and the ADP-ribose moiety is covalently bound to a single receptor in the EF-2 molecule, diphthamide, at N1, by an $\alpha$-glycosidic linkage.
The reaction goes almost to completion. Diphthamide, 2-[3-carboxamide-2-(trimethylammonia) propyl] histidine, located near the NH₂-terminal of the A chain, is a unique histidine derivative found only in EF-2 of eukaryotic cells and archaebacteria. The EF-2 is a GTPase; ADP-ribosylation of diphthamide prevents this activity, without altering the ability of EF-2 to bind GTP. Protein synthesis is arrested. About half of the diphtheria toxin molecules are associated non-covalently with a nucleotide, ApUp, similar in structure to NAD. ApUp blocks the nucleotide binding site on the A chain. Diphtheria toxoid, produced by treatment with formaldehyde or glutaraldehyde, has no enzymatic activity, although it binds to cells. It is internally crosslinked and does not separate into A and B subunits.

Little is known about the effects of diphtheria toxin on cells at the molecular level. There is apparently no specific target organ; arrest of protein synthesis eventually leads to cell death. Clinically, the organisms remain at the site of infection. The toxin produces necrosis of the local epithelium, which later becomes the characteristic diphtheritic membrane. Systemic effects are produced by entry of the exotoxin into the circulation. The myocardium and peripheral nerves are most affected.

**Enterotoxins**

These toxins, produced by several different organisms, act on the mucosal epithelium of the small intestine and produce a profuse watery diarrhea.

**Cholera Toxin**

Cholera toxin, secreted by *Vibrio cholerae*, is a protein composed of five identical B protomers (Mr 11,677) and one A protomer (Mr 27,215), held together noncovalently. The B units form a ring, with the A protomer in the center. The A protomer has a signal peptide at its N-terminal, composed of 18 hydrophobic aminoacid residues; the B protomers have signal peptides of 21 aminoacid residues. The A and B protomers are assembled into the complete toxin in the outer membrane of the organism (in contrast to EC-LT, which is assembled in the periplasmic space). The A protomer is cleaved by a protease into an A₁ amioterminal peptide and a smaller A₂ peptide (Mr about 5,000), which remains closely associated with the B protomer. A₁ and A₂ are joined by a disulfide link-
age. Both A and B protomers are encoded by chromosomal genes ctx A and ctx B, regulated by a gene tox R and possibly others. Protomer B is always produced in excess of A. Note, A₁ is an ADP-ribosyl transferase; B is responsible for binding the toxin to its surface-membrane receptor, A₂ assisting in this process.

The surface-membrane receptor on eukaryotic cells is the monosialoganglioside GM₁, which is widely distributed among different cell types. However, in the clinical disease, the organism is confined to the gastro-intestinal tract. The mode of internalization of the toxin has not been fully elucidated. It is believed that when bound to its receptor, the B protomers and A₂ bring A₁ close to the plasma membrane and, at the same time, create an opening for it to enter the cytoplasm. A₁ is then separated from the rest of the toxin by reduction of the disulfide bond. The target for A₁ is adenylate cyclase, which is membrane-bound on the cytoplasmic side of the membrane. Internalization of the toxin is not effected by RME. The enzyme transfers ADP-ribose from NAD⁺ to the regulatory protein Gₛ, as described previously, and produces an uncontrolled increase in cAMP. Cholera toxin acts like a hormone in its production of a second messenger, cAMP; however, unlike hormonal action, the production of the messenger is irreversible.

Cholera toxin attaches itself to receptors on the luminal surface of the intestinal epithelium, the effects of increased cAMP production depending on the cell. In villus cells, it has an antiabsorptive effect, that is, the inward flux of sodium and chloride ions from lumen to mucosa is inhibited; water absorption is therefore also prevented. The cause is believed to be phosphorylation of brush border components by a cAMP-dependent protein kinase. The cAMP also acts on the cells of the crypts of Lieber-kühn, in this case stimulating a flux of chloride and bicarbonate ions (accompanied by sodium ions) into the lumen, brought about by an increase in anion conductance of the luminal membrane. The toxin also ADP-ribosylates other substrates, but the consequences for human disease are not known.

**Escherichia coli**

**Enterotoxins**

These enterotoxins are responsible for “travelers’ diarrhea”. Some strains of *E. coli* colonize the mucosal surface of the small intestine and produce exotoxins EC-LT and EC-ST; a second group is enteroinvasive, like *Shigella* organisms, and multiply intracellularly, producing the same exotoxins. A third group, enteropathogenic *E. coli* (EPEC), produces a cytotoxin, similar to that produced by *Shigella dysenteriae* 1.

EC-LT is a heat-labile protein toxin, very similar to cholera toxin in its composition and properties. Like that toxin, it consists of an A subunit (Mᵣ 25,500 to 29,000) and five identical B pentamers of Mᵣ 11,800). The subunits are synthesized with signal peptides, assembled into the complete toxin in the periplasmic space and exported with outer membrane proteins in vesicles, as has been described previously. The A subunit consists of an A₁ chain (Mᵣ 21,000), which has enzymatic activity, and an A₂ chain, which fixes the A subunit to the ring of B pentamers and assists the B’s to bind to a specific receptor. There is considerable homology between these chains and the corresponding ones in cholera toxin; there is also antigenic cross-reactivity. The toxin is coded for by plasmid genes.

EC-LT has the same enzymatic action as cholera toxin. It is an ADP-ribosyl transferase, transferring ADP-ribose from NAD⁺ to the Gₛ regulatory protein of surface-membrane bound adenyl-
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ate cyclase, leading to an unregulated increase in cAMP production, and the same effects on salt and water absorption described for cholera toxin. EC-LT binds to the same G\textsubscript{M\textsubscript{1}} receptors on the brush border membrane of the small intestinal mucosa that bind cholera toxin; in addition, it binds to other receptors, probably galactoproteins.\textsuperscript{21}

EC-ST is a heterogeneous group of heat-stable, low molecular weight, peptides. There are two varieties, ST\textsubscript{A} and ST\textsubscript{B}, differing in methanol solubility. ST\textsubscript{B} is not an enterotoxin.\textsuperscript{27} ST\textsubscript{A} peptides have an M\textsubscript{r} of about 2,000 and consist of 18 to 19 aminoacid residues, of which six are cysteine. The toxin is tissue specific to the small intestinal brush border cells, where it binds to a high molecular weight protein, and not a ganglioside. It has no known enzymatic activity, but it produces an increase in eGMP in the cell by its stimulating effect on guanylate cyclase. This results in inhibition of the sodium coupled uptake of chloride by the villus cells, as occurs with cholera toxin. The mechanisms of internalization of the toxin and activation of guanylate cyclase are not known.

**Staphylococcus aureus Enterotoxins\textsuperscript{36,40}**

Some strains of this organism produce heat-stable enterotoxins, which cause severe diarrhea and vomiting. Seven different toxins have been described, A, B, C\textsubscript{1}, C\textsubscript{2}, C\textsubscript{3}, D, and E. They are all single chain polypeptides, with M\textsubscript{r}'s in the range of 26,000 to 30,000. While A is immunologically distinct, the others show considerable homology. C\textsubscript{1} has been sequenced. It has an M\textsubscript{r} of 27,500 and has 239 aminoacid residues. It is encoded by a plasmid. C\textsubscript{3} has 236 residues and an M\textsubscript{r} of 27,111. It is also encoded by a plasmid. The trio of C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{3} cross-react, but are not identical. A is encoded by a lysogenic phage. The targets and mode of action of these toxins are not known.

**Other Enterotoxins**

Some strains of Klebsiella, Enterobacter cloacae, Yersinia enterocolitica, and Shigella flexneri produce enterotoxins similar to ST\textsubscript{A}; Pseudomonas aeruginosa, Bacillus cereus,\textsuperscript{45} and Klebsiella produce an LT-like toxin. Some Salmonella organisms produce a cholera-like enterotoxin, which is heat-labile, of M\textsubscript{r} about 90,000 and have A and B protomers. Aeromonas organisms produce an enterotoxin different from LT, ST, and cholera toxin. Clostridium perfringens produces an enterotoxin similar to EC-LT, while C. difficile produces an enterotoxin stimulating guanylate cyclase activity. Vibrio vulnificus, V. fluvialis, and V. mimicus produce a cholera-like enterotoxin. Campylobacter jejuni produces enterotoxins resembling those of cholera and EC-LT.

**Shigella dysenteriae Type 1 Toxin\textsuperscript{3,12,32}**

This toxin (Shiga toxin) has cytotoxic, neurotoxic and enterotoxic actions, although its principal effect is cytotoxic. The toxin (M\textsubscript{r} 65,000) is composed of an A protomer (M\textsubscript{r} 32,000) and six or seven B protomers (M\textsubscript{r} of each about 5,000). The A protomer can be cleaved into A\textsubscript{1} and A\textsubscript{2} chains, linked by a disulfide bond. A\textsubscript{1} is an enzyme. The toxin is inactive when formed, but is activated in the target cell by proteolysis and reduction of the disulfide bond, liberating the free A\textsubscript{2} chain. The protomers are synthesized with N-terminal hydrophobic signal sequences and the toxin molecule is assembled in the periplasmic space of the organism. Toxin synthesis is phage encoded.

The toxin binds with high affinity to glycoprotein receptors on the surface membrane and probably enters the cell
by RME; however, the details are not known. Because the organism is intracellular in the intestinal mucosal cells, binding and internalization apply to its other target cells. The \( A_2 \) chain inhibits protein synthesis in the target cells by catalyzing the inactivation of the 60 S ribosomal subunit, by an unknown mechanism.

Enteropathogenic \( E. \) coli (EPEC)\(^{10} \) produce a toxin, similar to but not identical with Shiga toxin. The molecular weight is less. The toxin is composed of \( A \) and \( B \) protomers; \( A \) is nicked by proteases to give \( A_1 \) and \( A_2 \) chains, the former enzymatically inactivating the 60 S ribosome. Toxin production is encoded by a plasmid, which is easily transmitted to other \( E. \) coli.

**Neurotoxins**

The principal neurotoxins are tetanus and botulinus toxins. Although they are similar in many respects, their target organs are different and thus their clinical effects.

**Tetanus Toxin\(^{12,22,28,41} \)**

This is a protein exotoxin of \( M_r \) 150,000 produced by \( Clostridium tetani \), encoded by a plasmid. The toxin molecule consists of a light \( \alpha \)-chain, \( M_r \) 50,000 and a heavy \( \beta \)-chain, \( M_r \) 100,000, which consists of two fragments, \( \beta_1 \) and \( \beta_2 \). A disulfide linkage connects \( \alpha \) and \( \beta_1 \); there is an intrachain sulfide linkage in \( \beta_1 \). The toxin remains in the bacterium until its release by autolysis. It is activated by nicking by an intracellular protease before release, the \( \beta_1 \) fragment being separated. However, both chains are required for toxicity. Neither chain nor the whole molecule has demonstrable enzymatic activity.

The target structures for tetanus toxin are the peripheral nerve endings. The heavy chain binds to \( GD_{1b}, GT_{1b} \) and GQ-type gangliosides on neuronal membranes. These gangliosides are mainly neuronal in distribution and determine the specificity of the toxin. The nerve terminals have many binding sites, accounting for the high toxicity of tetanus toxin. The bound toxin is internalized, possibly by adsorptive pinocytosis, and undergoes retrograde axonal transport of motor, sensory and adrenergic fibers in smooth vesicles, cisternae and tubules, slowly reaching the post-synaptic dendrites. The toxin is selectively released from these and is taken up by the presynaptic nerve terminals. Some toxin is found in the lysosomes of the ganglion cells. It acts by preventing the release of acetylcholine, thus blocking inhibitory synapses in the spinal cord and causing a spastic paralysis.

**Botulinum Toxin\(^{12,22,28,43} \)**

There are eight antigenically different toxins, each produced by a different strain of \( Clostridium botulinum \). The organisms and the toxins are named A, B, C1, C2, D, E, F, and G. All except C2 are neurotoxins. C2 is a cytotoxin which induces hypotension, an increase in intestinal secretions, pulmonary hemorrhage and increased vascular permeability. The toxin is an ADP-ribosyl transferase, its substrate being \( \beta/\gamma \)-actin, preventing actin polymerization. Skeletal muscle actin is not affected.\(^1 \) C2 is secreted as two separate proteins, C2-I and C2-II, of respective \( M_r \)'s 50,000 and 100,000, which must be combined to produce a toxic effect. C2-I is the enzyme; C2-II binds the complete toxin to the cell and aids the translocation of the enzyme.

Toxins of \( C. \) botulinum are phage encoded, except for C2. Both C1 and D have a lysogenic phage, while the others have either a phage or a plasmid. The toxin is synthesized as a single chain polypeptide of \( M_r \) 150,000 to 160,000
and is nicked extracellularly by an endogenous protease after release by autolysis, to give a heavy (H) and a light (L) chain, similarly to tetanus toxin. The toxins act on cholinergic nerve endings, initially at the peripheral neuromuscular junctions and, after retrograde axonal ascent, to nerve endings for which acetylcholine is the transmitter. Botulinum toxin can reach any area of the central nervous system neuronally connected with the peripheral nerve endings. The blood brain-barrier is impermeable to the toxin. The heavy chain at its COOH end binds to gangliosides, probably G_{Tia} and others. The toxin is internalized at the neuromuscular junction, possibly by RME, but the true mechanism is not known. At the cholinergic nerve endings, it antagonizes events triggered by calcium ions that culminate in the release of acetylcholine, possibly by blocking calcium ion channels. The mechanism of axonal ascent is not clear.

Endotoxins

The outer membrane of gram negative bacteria contains lipopolysaccharide (LPS) as an integral part. The amphipathic macromolecule carries the antigenic determinants of O specificity. It is also an endotoxin, responsible for symptoms of a wide variety of septic and non-septic conditions. Lipopolysaccharides consist of a hydrophilic polysaccharide portion, which is responsible for its O- and R-antigenic properties and a covalently bound hydrophobic lipid component called lipid A, which is responsible for the endotoxic properties of LPS. The polysaccharide can be divided into an O-specific polysaccharide chain joined to a core heterooligosaccharide. The O-chain is made up of repeating oligosaccharide units, the diversity and arrangement of which differ in different bacteria and provide the O-specificity. The core is linked to the O-chain through one or more keto-deoxysaccharidic acids (KDO), forming the inner core, to sugar residues, often substituted by phosphate and phosphoylethanolamine. Rough (R) mutants lack the O-polysaccharide and part of the inner core.

Lipid A is covalently bound to the polysaccharide portion of LPS, through a KDO. Lipid A’s of various endotoxins resemble one another in their general structure. They have a phosphorylated glucosamine disaccharide backbone, with medium chain fatty acids attached to the 3 and 3’-hydroxyl and the amine radicals. The fatty acids have 12, 14, or 16 carbon atoms, are mostly saturated and some have hydroxyl groups. Lipid A is antigenic, antisera protecting against some of the effects of endotoxin, but not all. Endotoxicity resides in the complete LPS molecules, but the lipid A component plays an important part. The role of various structures in the molecule in producing toxic effects is under intense study, using newly available synthetic analogues.

The LPS is released from the bacteria, combined with outer membrane proteins, and other compounds, in outer membrane vesicles. Thus, although an endotoxin, it is found in the environment of the organisms and can be disseminated throughout the body. In the plasma, it is bound to high density lipoproteins and is slowly removed from the circulation by phagocytes. Most is eventually removed by the liver; some is degraded intracellularly in tissues. Endotoxin is also released within phagocytic cells as they process ingested bacteria and it may be produced locally by lysis of organisms.

The mechanism of endotoxin toxicity is not clear. Some cells are particularly sensitive to endotoxin, namely, monocyte, granulocytes, and endothelial cells. Endotoxin is also a potent activator of the complement system; activated com-
plement could be responsible for cell membrane damage and activation of the coagulation system. The entry of LPS into target cells is believed to involve binding to surface receptors, endocytosis and translocation of LPS through the cytoplasm to a receptor on the mitochondrial membrane. The mitochondrial proton gradient is destroyed, ADP and NADH accumulate in the cytosol leading to enhanced glycolysis and lactic acid production. The permeability of the cell membrane to calcium ions is increased; mitochondrial injury permits the accumulation of peroxides and superoxides and lipids are oxygenated producing prostaglandins. End results are release of lysosomal enzymes, active degradation of intracellular proteins and the new synthesis of replacements. Endogenous pyrogen is formed. The cellular events account for the observed effects of endotoxin toxicity: disturbed temperature regulation, changes in glucose and calcium concentrations, increased lactic acid production and lysosomal enzyme activities, disseminated intravascular coagulation, and circulatory hypotension.

Endotoxin is always being produced in the host by resident bacteria. Under normal conditions, the host defense mechanisms, many induced by the toxin in small amounts, clear it from the system in the liver or metabolically degrade it. Breakdown of the defense mechanisms and resulting endotoxemia may occur with septic lesions, liver dysfunction, severe hemorrhagic shock, adrenal insufficiency and other conditions. Endotoxin can be detected in the blood and other body fluids by use of the Limulus amebocyte lysate test (LAL). The lysate is clotted by endotoxin. A sensitive colorimetric modification of the LAL test is now available.

Although endotoxin is a product of gram negative bacteria, peptidoglycan, a basic structural component of the cell walls of all bacteria, can in large amounts produce endotoxin-like effects. Group A Streptococci, some Staphylococci and Mycobacterium tuberculosis produce endotoxin-like effects.

**Toxic Shock Syndrome**

This condition (TSS) is associated with strains of *Staphylococcus aureus* infected with group I bacteriophages, especially phage 29. On lysis, these bacteria produce an exotoxin, TST. This is a single chain polypeptide of M, 24,000. Other protein toxins, including staphylococcal enterotoxin B, have been found associated with TST. The role of these other toxins in producing TSS is not clear. However, it is known that endotoxin enhances the severity of TSS, probably by suppressing the immune system. Toxic shock syndrome is pyrogenic, particularly when following small amounts of endotoxin.

Toxic shock syndrome occurs mainly in menstruating women using tampons, but it also occurs in other women and to a lesser degree in males. The severe, often lethal, form of the disease is marked by multi-system organ damage and massive capillary vasodilation with intravascular fluid loss. Clinically, there are fever, a rash, desquamation of the skin of the palms and soles after one to two weeks, severe hypotension, and vomiting or diarrhea, myalgia, renal damage, liver damage, bone marrow depression and CNS involvement. Some of these findings are directly due to the toxic action on target cells, others are secondary to them. Mild forms of the disease occur.

**Bordetella pertussis toxins**

These have been studied intensively in recent years because of the high degree of reactogenicity of whole cell pertussis vaccines and the severity of some of the reactions. The major toxin is
pertussis toxin or pertussigen. In the past, this toxin was given many different names, each associated with one of its toxic effects in experimental animals. It is now known that these diverse effects are caused by one molecule, probably by one mechanism. Other toxins are adenylate cyclase toxin, dermonecrotic toxin, tracheal cytotoxin and lipopolysaccharide. Filamentous hemagglutinin, although not a toxin, assists the attachment of the organism to its target cells and is therefore a virulence factor.

Pertussigen is a hexameric protein of $M_r$ 117,000, the subunits being associated into an A-B dimer. The A protomer, $M_r$ 28,000, is an ADP-ribosylase. The heavier B-protomer is composed of four dissimilar subunits held together non-covalently and united by a second smallest subunit, which connects two pairs of dimers. The whole structure is reminiscent of cholera toxin. The B protomer binds the toxin to surface-membrane receptors on the target cell, possibly a ganglioside. The mechanism of internalization is not known. The A protomer transfers ADP-ribose from NAD$^+$ to the membrane bound 41,000 $M_r$ subunit of the inhibitory protein, $G_i$, of the guanine nucleotide-binding regulatory component of adenylate cyclase. The binding is enhanced by Mg$^{2+}$ and the 35,000 $M_r$ component of $G_i$. As a result, the response of the regulatory component to signals from cell surface receptors is attenuated or abolished and there is an unregulated increase in cAMP production when the cells are stimulated. Insulin secretion is potentiated through loss of $\alpha$-adrenergic inhibition. Other non-cAMP coupled receptor pathways are also blocked by pertussigen, e.g., the stimulation of phosphatidylinositol hydrolysis, arachidonate release and calcium mobilization. Immune effector cells are particularly affected. Similar mechanisms produce heightened sensitivity to histamine stimulation. Small amounts of this toxin may produce no overt effects, unless the subject is stressed and homeostatic mechanisms fail.

Adenylate cyclase toxin is secreted by the growing organism and its activity is increased a thousand times by eukaryotic calmodulin. In some way, this toxic enzyme enters eukaryotic cells and catalyzes the conversion of endogenous ATP to cAMP. The toxic enzyme has an $M_r$ of 190,000. Non-toxic adenylate cyclase, also produced by the organism, has an $M_r$ of 70,000.

Dermonecrotic toxin is heat-labile. It has an $M_r$ of 102,000 and has subunits of 24,000 and 30,000 $M_r$. It produces vasoconstriction of small blood vessels and local ischemia, when injected subcutaneously in mice. The mechanism of action is not clear, but the toxin does inhibit Na$^+$-K$^+$ ATPase activity.

Tracheal cytotoxin is a small peptide, probably derived from peptidoglycan. It binds specifically to ciliated cells and inhibits DNA synthesis. Its molecular mechanism of action is unknown.

Lipopolysaccharide differs from the LPS of gram negative bacteria. There are two immunologically distinct lipopolysaccharides, containing lipid A and lipid X, respectively. There are also two different oligosaccharide chains. The lipid X fraction has the characteristic endotoxic effects, but the lipid A fraction has little toxicity and is not pyrogenic. However, it has potent adjuvant and antiviral activities. Polypeptides associated with the lipopolysaccharides also have immunomodulating properties.

*Bacillus anthracis* 28

This organism produces three factors which combined are toxic: protective factor, edema factor and lethal factor. Protective factor plus edema factor form an A-B type toxin, which is responsible for the dermonecrotic effect of the toxin.
Edema factor is an inactive form of adenylate cyclase, which is activated after entry into the target cell by calmodulin, elevating cellular cAMP. Protective factor promotes entry of the enzyme into the cell. Lethal factor is lethal to animals when combined with protective factor. The mode of action is not known.

**Gas Gangrene Clostridia**

These organisms produce many toxins, most of which have not been well characterized. Some are enzymes, e.g., lecithinase, collagenase, neumaminidase, proteinase and hyaluronidase, deoxyribonuclease; others act on tissues, for example, the heart.

**References**