Review of Selected Bacterial Enterotoxins and Their Role in Gastroenteritis

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ABSTRACT

The study of enterotoxins began in earnest approximately 30 years ago with characterization of the cholera toxin. So far, more enterotoxins have been associated with Gram-negative rather than Gram-positive bacteria. These substances can be roughly divided into the cytotoxic variety, which primarly interfere with intestinal cell metabolism, and those which are cytotoxic, responsible for cell destruction.

Most cytotoxic enterotoxins activate cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) producing watery diarrhea, resulting from fluid and electrolyte flux. This typically results after binding to a toxin-specific receptor site on the small intestinal epithelium. Cytotoxic enterotoxins may interfere with protein synthesis in cells of the colonic epithelium and cause bloody, sometimes fatal dysentery.

As more enterotoxins are discovered, it is becoming apparent that such variables as anatomic site and mode of action may not, in fact, be definitive criteria for classifying these substances. As a result, no universally acceptable classification scheme has yet been devised. Moreover, the biochemical and physiological characteristics of many enterotoxins and their role in gastroenteritis and other disorders remain speculative.

In his excellent article on the evolution of molecular toxinology, Joseph Alouf points out that approximately 220 bacterial toxins of all varieties are now known.1 About half are produced by Gram-positive and half by Gram-negative organisms. The first to be discovered was the diphtheria toxin, described by Roux and Yersin at the Pasteur Institute in 1888. During that same period, Koch postulated the existence of the first enterotoxin from Vibrio cholerae. It was not until 1959, however, that the actual heat-labile cholera toxin was specifically identified, which ushered in the study of enterotoxic substances in particular.

Since that time, it has been found that enterotoxins are a group of bacterial protein exotoxins which act specifically on the gastrointestinal epithelium of humans and certain animals. The primary clinical manifestations of enteroinfection are diarrhea and vomiting. These substances may be produced in foods prior to ingestion, as exemplified by staphylococcal enterotoxin B, or elaborated in the gut, such as that produced by Vibrio cholerae. Several genera of
bacteria produce enterotoxins with more being discovered each year. This review will seek to provide a summary of a few of the more prominent enterotoxins, their sources, and modes of action in the human gastrointestinal tract.

One problem with the study of enterotoxinology derives from the lack of a suitable classification scheme for these substances. Our knowledge is incomplete in this respect, but a few general observations can be made. It appears that more enterotoxins are produced by Gram-negative rather than Gram-positive bacteria. Most of these are rather large molecules, greater than MW 70,000, and a preponderance of them exert their primary effect via activation of adenylate cyclase (or guanylate cyclase), in the intestinal epithelium of the small bowel (table I).

Guerrant provides an informative discussion of enterotoxins, which have been divided on the basis of their neurotoxic, cytotoxic, or true enterotoxic properties. However, this approach can be confusing since certain enterotoxins, such as that produced by Bacteroides fragilis, may exert multiple gastrointestinal effects. Guerrant does offer a valuable scheme for the general delineation of enteric infections, which he divides into (1) those produced by true enterotoxins, occurring primarily in the proximal small intestine (i.e., Vibrio cholerae), (2) those of the distal small intestine, with questionable enterotoxin involvement, producing the true enteric fevers (i.e., Salmonella typhi), and (3) those of the colon, some of which involve enterotoxins, which produce classical dysentery (i.e., Shigella dysenteriae).

In addition, it is often difficult to correlate physiologic mechanisms with anatomic location. Certain genera, such as Escherichia coli, clearly produce multi-

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**TABLE I**

Selected Enterotoxin-Producing Bacteria: Gram Stain Characteristics, Molecular Weight, Type, Site, and Mode of Action of Enterotoxins

<table>
<thead>
<tr>
<th>MW Enterotoxin</th>
<th>Type/Site*</th>
<th>Mode of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-Negatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>92,000</td>
<td>N/S</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli LT</td>
<td>91,000</td>
<td>N/S</td>
</tr>
<tr>
<td>Enterotoxigenic E. coli ST</td>
<td>2,000</td>
<td>N/S?</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>90,000</td>
<td>N/S?</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>10,000</td>
<td>N/S</td>
</tr>
<tr>
<td>Aeromonas species</td>
<td>15,000</td>
<td>N/S</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>70,000</td>
<td>X/L</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>N/S?</td>
<td>N/S?</td>
</tr>
<tr>
<td>Klebsiella species</td>
<td>N/S?</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>N/S?</td>
<td></td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>N/L?</td>
<td>NOT FULLY CHARACTERIZED</td>
</tr>
<tr>
<td>Enterohemorrhagic E. coli</td>
<td>X/L?</td>
<td></td>
</tr>
<tr>
<td>Enteropathogenic E. coli</td>
<td>X/L?</td>
<td></td>
</tr>
<tr>
<td>Enteroinvasive E. coli</td>
<td>X/L?</td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>X/L?</td>
<td></td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>20,000</td>
<td>XN/SL?</td>
</tr>
</tbody>
</table>

**Gram-Positives**

| Staphylococcus aureus A-P | 22,000- 34,100 | N/S | Unclear |
| Clostridium perfringens | 35,000 | X/S | Inhibition of protein synthesis |
| Clostridium difficile toxin A | 550,000 | X/L | Colonic fluid accumulation? |
| Clostridium difficile toxin B | 350,000 | X/L | Unclear |
| Bacillus cereus | 55,000 | X/? | Activation of cAMP |

* N = Cytotoxic Enterotoxin  X = Cytotoxic Enterotoxin  S = Small Intestine  L = Large Intestine

**cAMP** = cyclic adenosine monophosphate

**cGMP** = cyclic guanosine monophosphate
ple enterotoxins, each of which has its preferred site of action. Betley et al and others choose to classify enterotoxins according to their general mode of activity. Thus, cytotoxic enterotoxins, exemplified by those of *V. cholerae*, enterotoxigenic *E. coli*, and *Staphylococcus aureus* are responsible for alterations of cellular metabolism, producing the watery diarrhea characteristic of small bowel electrolyte flux. Cytotoxic enterotoxins, such as those produced by *S. dysenteriae*, enteropathogenic *E. coli*, and *Clostridium difficile*, exert destructive cellular effects associated with inflammatory colonic diseases.

Cytotoxic enterotoxins comprise the larger group. Many members of this "family" of substances share certain structural, functional, and immunological properties with the enterotoxin produced by O group 1 *V. cholerae* strain 569. These enterotoxins are products of strains of *E. coli*, *Salmonella*, other *Vibrio* species, *Aeromonas*, *Campylobacter jejuni*, and probably others. They are typically elaborated after colonization of the small bowel. Several excellent reviews have been published describing the mode of action of these substances. Only the high points will be touched upon here.

Basically, *V. cholerae* enterotoxin, the prototype of this "family", is composed of three subunits, $A_1$ (MW 29,000), $A_2$ (MW 5,500), and B (MW 11,500). Subunit B (a pentamer) is responsible for binding to an enterotoxin-specific mucosal cell surface receptor, ganglioside GM1. The $A_2$ subunit acts in the enterotoxin internalization process, while subunit $A_1$ enzymatically transfers adenosine diphosphate (ADP)-ribose from nicotinamide adenine dinucleotide (NAD) to a guanosine triphosphate (GTP)-$G_s$ regulatory protein complex of the adenylate cyclase system. The ribosylated $G_s$ protein-GTP complex then interacts with a cytoplasmic membrane bound catalytic component to promote cAMP formation from adenosine triphosphate (ATP). Normally, the regulatory protein dissociates following catalysis, and subsequent hydrolysis of GTP to guanosine diphosphate (GDP) deactivates cyclase activity. The toxin-induced ADP-ribosylation of the regulatory protein, however, prevents GTP hydrolysis, and the cyclase process is thus perpetuated resulting in sustained increased cAMP levels, which in turn promote ion flux changes in the small bowel mucosal lining, along with characteristic watery diarrhea.

Traveler's diarrhea is most often caused by certain enterotoxigenic strains of *E. coli*. Two distinct enterotoxins are produced. One is inactivated by heating at 60°C for 30 minutes, while the other retains its activity after boiling for 30 minutes. Both toxins are coded for by transmissible plasmids. The heat-labile enterotoxin (LT) is MW 91,000, and is related structurally, functionally, and antigenically to the cholera enterotoxin. This toxin also binds to the GM1 cholera toxin receptor and activates mucosal cell adenylate cyclase via ADP-ribosylation of the same MW 42,000 GTP-bound regulatory protein.

The *E. coli* heat stable enterotoxin (ST) is a much smaller molecule, with MW of 2,000, and bears no antigenic cross-reactivity with LT or cholera toxin. These enterotoxins may be produced by multiple strains of *E. coli*, each strain capable of producing several types of ST, which differ in their methanol solubility and host species specificity. Several cases of traveler's and childhood diarrhea owing to ST have been described by Greenberg and Guerrant. At least one type of stable toxin, ST$_a$, exerts its effects through activation of guanylate cyclase with subsequent increased levels of cGMP accompanied by watery diarrhea. It is tempting to equate this system with that of LT and
cholera toxin, although the mechanism of guanylate cyclase stimulation remains unclear. Another *E. coli* stable toxin, STₐ is structurally similar to STₐ, but its role in human disease is vague, and its mechanism of action unknown. Considering the small size of ST molecules, it is much more probable that these act stoichiometrically rather than catalytically.²³

Several other bacterial genera produce enterotoxins structurally and physiologically similar to *E. coli* LT or ST.³ Baloda et al have described a heat liable enterotoxin from strains of *Salmonella enteritidis* and *S. typhimurium.*² This substance binds to the GM₁ ganglioside receptor in the small intestine and may act similar to cholera toxin.

Okamoto et al have described a MW 10,000 enterotoxin produced by *Yersinia enterocolitica* that closely resembles *E. coli* ST.³⁰ It operates via activation of guanylate cyclase and cGMP. The significance of this toxin in clinical disease is unclear.⁶

Certain strains of *Aeromonas* species produce cytotoxic enterotoxins, at least one of which is MW 15,000 and has been shown to be heat stable at 56°C.²⁰,²⁶ This substance is structurally and immunologically unrelated to the aforementioned “family” of cholera-like enterotoxins, although its mode of action seems to favor activation of cAMP.²¹ Although this enterotoxin can promote diarrhea, or occasionally cholera-dysentery-like syndromes, its relationship to clinical disease remains undefined.⁵,³⁷

Other genera demonstrating cytotoxic enterotoxic potential include *Enterobacter, Klebsiella, Shigella flexneri,* and possibly others.³ Details concerning the clinical significance and modes of action of enterotoxins from these organisms are currently incomplete.

*Staphylococcus aureus* produces a variety of enterotoxins, MW 22,000 to 34,100, serologically grouped from A through F. Typically, the enterotoxin is produced in food substances prior to consumption. One to six hours after introduction into the small intestine, the cytotoxic enterotoxin acts in an unspecified manner to produce abrupt watery diarrhea, nausea, and vomiting by disrupting fluid and electrolyte balances.¹⁴ The basis for this appears to be one of water absorption inhibition rather than electrolyte flux.³

Each enterotoxin is a heat stable single chain protein molecule, some of which cross react serologically.³ Recently, Crass and Bergdoll have implicated staphylococcal enterotoxins in toxic shock syndrome.⁷,⁸ Enterotoxin A was associated with coagulase-negative staphylococci, while enterotoxin B was more typically a product of *S. aureus.* Gastroenteritis was not a significant clinical feature with these patients, and the role of these enterotoxins in toxic shock syndrome remains speculative.

Cytotoxic enterotoxins include intestinal necrotic agents produced by a variety of bacteria either before or after ingestion of contaminated food or water. They manifest in terms of bloody, sometimes fatal diarrhea. Many of them can enter the blood stream and produce toxic effects elsewhere in the body. Typically, their actions are associated with intoxication of the distal small bowel and colon.

As demonstrated by Eiklid and Olsnes, the enterotoxin of *Shigella dysenteriae* Type 1, often termed Shiga toxin, or Vero toxin, is also cytotoxic and neurotoxic.¹⁰ This substance operates primarily by inactivating the 60S ribosomal subunit of colonic epithelial cells, thereby preventing protein synthesis.²³,³¹ The resulting cytotoxicity produces serious or fatal colitis and dysentery. The Shiga toxin is a dichain molecule, MW 70,000, and probably exists as a periplasmic protein of two main subunits. The A subunit (MW 38,000) is enzymatically active and requires lysosomal proteolytic cleavage.
into A₁ and A₂ fragments for expression of full enzymatic activity. Inactivation of the 60S ribosomal subunit is accomplished by the A₁ fragment subsequent to binding by six or seven B subunits (MW 5,000). The A₂ fragment probably acts in the toxin internalization process. Both A and B subunit genes are encoded by a single phage type H19B. The Shiga toxin will not be produced in vitro in the presence of large amounts of iron. Possibly, for this reason, toxin production has often gone undetected owing to suppression by iron-rich culture medium.

Several cytotoxic Shiga-like enterotoxins have been reported by strains of enteropathogenic, enteroinvasive, and enterohemorrhagic E. coli, Shigella flexneri, Salmonella typhimurium, Vibrio cholerae, and Vibrio parahemolyticus. These substances are encoded by a group of closely related bacteriophages. They are very similar in most respects, but some differ in molecular weight. Of considerable clinical importance among these is the enterotoxin produced by enterohemorrhagic E. coli, responsible for epidemic hemorrhagic colitis in the United States. One of the possible complications of this disorder is the hemolytic uremic syndrome, consisting of acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. The mechanism of action for the enterotoxin in this disorder is unclear.

Clostridium perfringens, one of the most frequent causes of food poisoning in the United States, produces a cytotoxic enterotoxin, MW 35,000. This substance inhibits glucose uptake, energy production, and macromolecular synthesis within the distal small intestine. Levels of cAMP remain static although the enterotoxin causes a marked reduction in oxygen consumption by epithelial mitochondria. These effects are responsible for structural damage and produce alterations in fluid and electrolyte balances. Inhibition of protein synthesis occurs after binding of the hydrophilic moiety of the toxin to cellular receptors, with release of the hydrophobic portion into the cell. The toxin is unique in at least two other ways: direct cytotoxic alteration of cellular membranes, and extremely rapid action, usually causing damage within 30 minutes of exposure.

Clostridium difficile produces antibiotic-associated pseudomembranous enterocolitis as the result of the combined effects of two distinctly large hydrophobic enterotoxins. Toxin A, MW 550,000, possesses some cytotoxic activity and promotes colonic fluid accumulation without activating adenylate cyclase. Toxin B, MW 350,000, is much more cytotoxic than toxin A, and causes necrosis and hemorrhage of the mucosa. Both toxins are capable of entering the peripheral circulation where their effects can prove lethal.

Bacillus cereus produces a cytotoxic enterotoxin MW 55,000, causing diarrhea, fluid accumulation, and intestinal necrosis 8 to 16 hours after ingesting contaminated food. The site and mode of action remain unclear, but probably involves activation of cAMP. In addition, an emetic factor MW <5,000 has been identified. Its role in B. cereus enterointoxication is conjectural.

Enterotoxin produced by certain strains of Bacteroides fragilis generates both cytotonic as well as cytotoxic effects. Recently, Myers and coworkers recovered toxigenic strains of this organism from the stool of eight individuals. Patients in this study ranged in age from infancy to 69 years. Two adult patients demonstrated predominantly watery diarrhea, suggesting cytotonic etiology. Four of six children, however, manifested frank or occult blood in stool specimens, indicating possible colonic cytotoxicity. These disorders appeared to be self-limiting and of one to four weeks duration.
The *B. fragilis* enterotoxin, while not yet fully characterized, is heat-labile and probably of the MW 20,000 range. It is produced after colonization of the distal small bowel and/or colon, but its mode of action is unknown. Unfortunately, no acceptable means for distinguishing toxicogenic from nontoxicogenic strains of *B. fragilis* in the clinical laboratory have yet been devised.

In summary, although more enterotoxins have been thus far recovered from Gram-negative rather than Gram-positive organisms, one of our largest problems remains classifying these substances. For example, one of the most recently described enterotoxins, that produced by certain strains of *B. fragilis*, seems to possess an age-dependent element, possibly producing cytotoxic symptomatology in adult patients, but generating cytotoxicity in young children. It is therefore apparent that a suitable system of enterotoxin classification is needed in order to elucidate the multiple roles played by these substances in gastroenteritis and other disorders.

References

23. **Middlebrook, J. L. and Dorland, R. B.**: Bac-