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Shiga Toxin (Stx) Classification, Structure, and Function

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Abstract

Shiga toxin (Stx) is one of the most potent bacterial toxins known. Stx is found in *Shigella dysenteriae* 1 and in some serogroups of *Escherichia coli* (called Stx1 in *E. coli*). In addition to or instead of Stx1, some *E. coli* strains produce a second type of Stx, Stx2, that has the same mode of action as Stx/Stx1 but that is antigenically distinct. Because subtypes of each toxin have been identified, the prototype toxin for each group is now designated Stx1a or Stx2a. The Stxs consist of two major subunits, an A subunit that joins noncovalently to a pentamer of five identical B subunits. The A subunit of the toxin injures the eukaryotic ribosome, and halts protein synthesis in target cells. The function of the B pentamer is to bind to the cellular receptor, globotriaosylceramide, Gb3, found primarily on endothelial cells. The Stxs traffic in a retrograde manner within the cell, such that the A subunit of the toxin reaches the cytosol only after the toxin moves from the endosome to the Golgi and then to the endoplasmic reticulum. In humans infected with Stx-producing *E. coli* (STEC), the most serious manifestation of the disease, the hemolytic uremic syndrome or HUS, is more often associated with strains that produce Stx2a rather than Stx1a, and that relative toxicity is replicated in mice and baboons. Stx1a and Stx2a also exhibit differences in cytotoxicity to various cell types, bind dissimilarly to receptor analogs or mimics, induce differential chemokine responses, and have several distinctive structural characteristics.

Shiga toxin (Stx) is one of the most potent biological poisons known. Stx causes fluid accumulation in rabbit ileal loops, renal damage in mice, rabbits, greyhounds, and baboons, and is lethal to animals upon injection. However, humans encounter Stx as a consequence of infection with *Shigella dysenteriae* type 1 or certain serogroups of *E. coli* such as the O157:H7. There are two immunologically distinct groups of Stxs and this review will discuss toxin classification, structure and function, and the virulence associated with Stx-producing *E. coli* (STEC).

OVERVIEW

S. dysenteriae and the Stx were identified in the 19th century by Drs. Neisser and Shiga (1) and Conradi (2). Approximately 80 years later the same toxin (now called Stx1 to distinguish it from the toxin produced by *S. dysenteriae*) was found in a group of *E. coli* isolates. These bacteria caused bloody diarrhea and a serious sequelae, the hemolytic uremic syndrome (HUS), a condition characterized by thrombocytopenia, hemolytic anemia, and kidney failure (3, 4). Some *E. coli* strains were later shown to produce a highly related toxin, Stx2, that has the same mode of action as Stx/Stx1 but that is immunologically distinct. The Stxs (also known as Vero toxins, and previously as Shiga-like toxins) are a group of

bacterial AB5 protein toxins of about 70 kDa that inhibit protein synthesis in sensitive eukaryotic cells. Protein synthesis is blocked by the Stxs through the removal of an adenine residue from the 28S rRNA of the 60S ribosome. This *N*-glycosidase activity of the toxin resides in the A subunit. The pentamer of identical B subunits mediates toxin binding to the cellular receptor globotriaosylceramide (Gb3). Additional commonalities between the Stx groups are that the subunit genes are encoded in an operon with the A subunit gene 5' to that of the B subunit, that the *stx* operon is usually found within the sequence for an inducible, lysogenic, lambda-like bacteriophage, and that the toxins utilize a retrograde pathway to reach the cytoplasm. Differences between the two toxin groups include the fact that the genes for *stx/stx1a* are repressed by the Fur when high levels of iron are present (5–7), and that *E. coli* strains that encode *stx2* are epidemiologically linked to more severe disease than those that carry *stx1* (8, 9).

TYPING AND NOMENCLATURE

Although the prototype *E. coli* Stxs from each main group, Stx1 and Stx2 (now called Stx1a and Stx2a for distinction in the nomenclature from other toxin subtypes (10)), are the most common types found in association with disease from their respective groups, subtypes of each toxin exist, listed in Table 1. Toxin subtypes were originally only recognized when differences in biological activity and/or immunoreactivity could be demonstrated. However, as many new Stx-producing *E. coli* (STEC) were isolated and the toxin genes from those strains were sequenced, it became difficult to know if any differences found between the newly isolated gene and the prototype *stx2a* should result in the designation of another toxin subtype. Therefore, a phylogenetic analysis of *stx* sequences was undertaken and a PCR typing scheme developed that enables the assignment of a toxin to a particular subtype (10).

Stx/Stx1 subtypes

To date no variants of Stx as produced by *Shigella* have been described, but Stx is occasionally found in *S. sonnei* and type 4 *S. dysenteriae* (11, 12). Only two variants of Stx1a have been identified: Stx1c and Stx1d. Both Stx1c and Stx1d can be distinguished immunologically from Stx1 (13, 14). Stx1c and Stx1d are rarely found in human disease, and when associated with STEC isolated from patients, are linked with a mild disease course (15, 16).

Stx2a subtypes

The first Stx2a toxin variant identified as important for human disease, Stx2c, exhibits reduced cytotoxicity on Vero cells and reacts differently than Stx2a to some monoclonal antibodies (17). Another Stx2a variant, Stx2d (Stx2dactivatable), was identified because incubation with elastase from intestinal mucus increases the Vero cell cytotoxicity of the toxin (18, 19). The activatable Stx2d is associated with the most serious manifestation of STEC infection, the HUS (20). Both Stx2c and Stx2d show reduced cytotoxicity for Vero cells due to two amino acid (aa) differences in the B subunit but Stx2d is as toxic as Stx2a when injected into animals (21). Moreover, strains that produce Stx2d are highly virulent in a streptomycin-treated mouse model of infection (18, 22). In contrast, Stx2c is reported to show reduced toxicity compared to Stx2 when injected into mice (23). A different Stx2a

subtype that was originally also named Stx2d, now known as Stx2b, is not activatable and is associated with mild disease (24, 25). Stx2e, Stx2f, and Stx2g are associated with animal STEC. Of the latter toxins, only Stx2e is associated with disease in the animal host; this toxin causes edema disease of swine, a rare, but serious neurological disorder that is frequently fatal (26).

SHIGA TOXIN STRUCTURE

The mature A subunit of Stx/Stx1a consists of 293 aa while the Stx2a A chain is 4 aa longer at the C terminus. The active site aa of the toxin is the glutamic acid at position 167 (27). A trypsin sensitive region (aa 248–251) allows the A subunit to be cleaved asymmetrically into an A₁ subunit and A₂ peptide held together by a disulfide bridge, see illustration Fig. 1. The enzymatic activity of the toxin resides within A₁ while the A₂ peptide tethers A₁ to the binding moiety, and further, threads through the B pentamer. For Stx2a the A₂ peptide, in addition to maintaining holotoxin structure, appears to partially block the site three Gb3 binding site (discussed further below) (28), and for Stx2d, is crucial for the activation phenotype, as the final two aa of the A₂ are cleaved when the toxin is treated with elastase (29). The pentamer of identical B subunits (each subunit = 69 aa for Stx/Stx1a, 71 aa for Stx2a) enables the toxin to find target cells that express surface Gb3. The crystal structures of the Stx B pentamer (30), Stx (31), Stx2a (28), Stx2a complexed with adenine (32), and the solution structures of the Stx1a B pentamer alone (33) or with the trisaccharide moiety from Gb3 (34) or a Gb3 analog (35) have been reported. A ribbon diagram of the crystal structure of Stx is shown in Fig. 2.

Genetic analyses of the Stx A subunit

Besides the active site glutamic acid, colored red in Fig. 2., other A subunit aa residues that contribute to the full enzymatic function of Stx, colored pale blue in Fig. 2, include N75, Y77, Y114, R170, and W203 (W202 in Stx2) (27, 36–38). An analysis of truncated A₁ fragments of Stx1a in yeast confirmed that residues within aa 1–239 are required for full enzymatic activity of the toxin, while the aa from 240–245 (and perhaps up to 251) are necessary for translocation of the A₁ from the endoplasmic reticulum (ER) to the cytosol (39). The authors of the latter study hypothesized that it is the general structure of the region between aa residues 240 and 251 that are recognized by anER mechanism that directs proteins from the ER into the cytosol. To define regions of Stx1a and the ribosome that interact, Garipey's group used yeast-two-hybrid and pull-down experiments and identified three ribosomal proteins that interact with the A₁ subunit of the toxin and further showed that a conserved peptide from two of the ribosomal proteins inhibited Stx1a activity in vitro (40). They also found that the region from R170 to L233 (purple in Fig. 2) in Stx1a is important for ribosome interaction (41).

Stx AND CELL BINDING

As noted previously, the Stxs bind to the glycosphingolipid Gb3, a molecule comprised of a lipid or ceramide component and a trisaccharide of (α -gal(1→4)- β -gal(1→4)- β -glc) (42–44). Cell lines and mice deficient in Gb3 are insensitive to the Stxs (45–47). The normal cellular function of Gb3 is not known; however, individuals with excess Gb3, a condition called

Fabry's disease (48), exhibit kidney disease among other symptoms. When challenged with Stx2a, a mouse model of Fabry's disease exhibits an elevated LD₅₀ (49), perhaps due to mistargeting of the toxin to multiple sites of the body rather than the kidney, or to altered cellular trafficking due to changes in Gb3 subspecies populations. Stx1a and Stx2a interact with globotetraosylceramide (Gb4) in addition to Gb3, though weakly (50), while Stx2e binds Gb4 in preference to Gb3 (51, 52). An early report of a possible protein receptor for the Stxs (53) has not been substantiated in the literature.

Gb3 clusters within detergent insoluble portions of membranes called "lipid rafts" which also contain cholesterol and the cholera toxin receptor monosialotetrahexosylganglioside or GM1 (54, 55). The interaction of the Stx1a B subunit with HeLa cells causes a 2.5-fold increase in Gb3 present in the lipid raft domains (56), a result that suggests that cell binding by the B pentamer may promote stronger or additional toxin/cell interaction by recruiting more receptors to the rafts. Several reports indicate that the fatty acid chain length of the lipid component of Gb3 as well as the saturation state of the lipid also influence toxin binding (57, 58). The presence of cholesterol in the lipid rafts has been reported to increase toxin binding (50). Conversely, another group found no role for cholesterol in cell binding by the B subunit of Stx1a, but they did find that reductions in the cholesterol content of cells resulted in a decrease in uptake of the B pentamer (55). More recently, Lingwood's group found that extraction of cholesterol from adult renal tissue sections enhanced Stx binding to glomeruli (59), a result that confirms the co-localization of Gb3 and cholesterol molecules. However, it may be difficult to dissect a possible role for cholesterol because Gb3 and cholesterol are both present in the lipid rafts, and alteration of either component may disrupt the integrity of the rafts. Taken together, these studies paint a picture of a complex interaction of the toxin with the host cell receptor, and suggest that the Stxs may interact differently with cells based on the nature of the receptor environment in the cell membrane.

The high affinity of the Stxs for Gb3 is likely due to the presence of at least two and up to three Gb3 binding sites per B monomer (two of the binding sites are present between adjacent monomers) as demonstrated by modelling studies and the crystal structure of the Stx B pentamer complexed with a Gb3 trisaccharide analog (35, 60). However, precise measurements of the binding affinity of the Stxs for Gb3 are difficult because Gb3 is not soluble. Therefore, soluble forms of the trisaccharide or trisaccharide analogs, or immobilized versions of the trisaccharide, Gb3, or Gb3 analogs are used to measure toxin/receptor interaction. When the interaction between the Stx1a B pentamer and the Gb3 trisaccharide was assessed in solution, binding site 2 (present within each monomer) was found to be the most highly occupied; site 1 was less engaged, and no interaction with site 3 was detected (34). Another study that also examined the interaction of the B pentamer with the Gb3 oligosaccharide component found that, at low concentrations of ligand, only site 2 was occupied though the authors of that study acknowledged that Stx binding is likely to be "polyvalent" at the cell surface (61). A mutational analysis of the three putative Gb3-binding sites in Stx1a confirmed the role of sites 1 and 2 for toxin/receptor interaction (62). A mutation within site 3 (W34A) reduced binding of the holotoxin to Gb3, although the mutant toxin had the same overall affinity for Gb3 as did Stx1a. Another group found that Gb3-binding site 2 alone is not sufficient to confer high avidity binding of the toxin to Gb3, and that optimal binding to the receptor required all three sites (63). Although these latter studies

on the number of functional Gb3 binding sites per toxin molecule were performed with Stx/Stx1a, the presence and primary importance of site 2 was confirmed for Stx2a (64). In addition, that site 1 contributes to the Vero cell toxicity phenotype of the Stx2a group is demonstrated by the fact that a single aa change in the Stx2d B subunit in the site 1 binding region renders that toxin as potent for Vero cells as Stx2a (21). Finally, a point in favor of the polyvalent nature of Stx binding is that Gb3 mimics with higher densities and clustering of the Gb3 trisaccharide (up to 18 copies) demonstrate higher affinities for both Stx1a and Stx2a (65, 66).

The question of how Stx enters the host from the site of pathogen colonization in the intestine remains, due to reports that colonic tissue lacks Gb3 (67). Macropinocytosis is a possible alternate mechanism for the uptake of Stxs into cells that do not express Gb3 (68). Polarized colonic T84 cells (which lack Gb3) nonetheless take up the Stx1a B pentamer in a way that is partially blocked by inhibitors of clathrin-dependent endocytic processes (68). However, such inhibition of the macropinocytic pathway does not reduce the amount of B subunit that is transcytosed through the monolayer, a finding that suggests that no matter which entry mechanism the Stx uses to enter these cells, the toxin can reach the basolateral side. That both Stx1a and Stx2a cross polarized T84 cells without disrupting the monolayer (46, 69, 70) or inhibiting protein synthesis has also been demonstrated (71). Therefore, it may be that some Gb3-negative intestinal cells allow systemic delivery of toxin in the absence of receptor. However, we and others have shown that incubation of intestinal cells with butyrate increases the sensitivity of intestinal cell lines to the Stxs (72, 73), and we further found that expression of Gb3 on intestinal cells is exquisitely sensitive to the removal of that gut metabolite (74). This latter finding suggests that previous measurements of Gb3 levels on intestinal tissues may be underestimates if butyrate was removed prior to Gb3 detection. In support of the possible presence of functional Gb3 receptors on intestinal tissue is the finding that incubation of pediatric intestinal explants with Stx2a showed cell extrusion from tissues taken from both ileum and colon (71). The damage observed in the latter study was specific as pre-incubation of the toxin with antiserum to Stx2a ameliorated the tissue injury. Furthermore, both Stx1a and Stx2a bind to Paneth cells when overlaid onto normal or inflamed pediatric duodenal tissues collected during endoscopy (75). We also found that that toxin overlaid into normal adult tissue binds colonic tissue (72). Finally, Malyukova et al. identified both Stx1a and Stx2a in intestinal tissues (epithelial cells and lamina propria) from O157-infected patients (68), although Gb3 could not be found in those intestinal epithelial cells (68). However, it may be that the Gb3 is masked in such tissues by cholesterol as described earlier, or that the expression of Gb3 was reduced by removal of butyrate when those samples were processed.

Stx/Gb3 interaction leads to uptake of the toxin/receptor complex through clathrin-dependent or -independent mechanisms. However, the clathrin-dependent process of entry appears to be the most common mode of uptake of the toxin/receptor complex (76). One perhaps surprising finding is that the A subunit of the Stx is involved in toxin uptake: more holotoxin is taken up via the endocytic pathway when the holotoxin binds to a cell as compared to B pentamer alone (77). The latter study illustrates the importance of confirming with the holotoxin findings based on the B pentamer alone. In clathrin-independent uptake, the Stx B subunit induces tubular-shaped invaginations within the HeLa cell plasma

membrane, the function of which are not clear (78). Curiously, a mutation in Gb3 binding site 3 of the B subunit prevented formation of the invaginations (78), although the pentamer still bound to the cells.

RETROGRADE TRAFFICKING OF THE Stxs

Stx was first shown to utilize a retrograde pathway to reach the cytosol more than 20 years ago in the human epidermal carcinoma cell line A431 which had been sensitized to the toxin by butyric acid treatment (79). An outline of the retrograde pathway used by the Stxs is shown in Fig. 3, and summarized as follows: after binding to Gb3, the toxin/receptor complex enters early endosomes, then traffics to the Golgi, and finally the ER (for a review see (80)). The protease sensitive site of the toxin A subunit is nicked either within the intestinal mucus (18) or within the Golgi (81); however, the nicked toxin retains the AB₅ structure because of a disulfide bond between the A₁ and A₂ chains. The disulfide bond is reduced once the toxin gets into the ER, and only the toxin A₁ subunit enters the cytosol. The Stx receptor, Gb3, is required for the toxin/receptor complex to move through the retrograde pathway, as toxin taken up by a nonreceptor-mediated mechanism or in cells depleted of glycosphingolipids do not reach the Golgi, though the complex does reach endosomes and possibly the ER (56, 70, 82). Many cellular proteins have now been identified that are involved in the Stx retrograde pathway (reviewed elsewhere (80, 83)). An unfolded toxin A₁ chain exits the ER, apparently by subverting the ER-associated protein degradation (ERAD) pathway, to reach the target ribosomes (84, 85). The A₁ then removes an adenine from the alpha-sarcin loop in the 28S ribosomal subunit. The injured ribosome no longer associates with elongation factor 1 (86, 87), and protein synthesis is halted. Although much of the work describing the retrograde pathway was done in epithelial cells, the primary target cell for the Stxs is the endothelial cell, discussed further below, and the Stx B pentamer has been shown to enter the retrograde pathway in mesangial and glomerular vascular endothelial cells as well (88).

ACTIONS OF Stx IN TARGET CELLS

Numerous studies show that Stx inhibits protein synthesis in target cells and that active site mutants of Stx are no longer cytotoxic. Furthermore, a single molecule of Stx may be sufficient to kill a cell (85). Recently, however, the effects that the toxin has on cell signal transduction and immune modulation have begun to be explored (reviewed (89, 90)). Stx-mediated damage to the ribosome induces a response in cells called a “ribotoxic stress response” which is both pro-inflammatory and pro-apoptotic (see review (89)). The Stxs are also associated with an unfolded protein response that comes about through stress on the ER, and the result of which may also be apoptotic (reviewed (89, 91)). Evidence that Stx and STEC are associated with apoptosis came from rabbit studies in the mid-1980s (92, 93). Since that time, the molecular mechanisms of apoptosis due to the Stxs has been elucidated further (see review (91)).

Stx1a COMPARED TO Stx2a

Stx1a is about 10-fold more cytotoxic to Vero cells than Stx2a; however, the reverse is true in mice: Stx1a is 100–400-fold less lethal to mice than Stx2a, even though the toxins exhibit

equivalent enzymatic activities/ng protein *in vitro* (94, 95). Epidemiological data from human disease indicate a stronger association with severe disease for STEC that produce Stx2a than Stx1a alone (96–98). So an important question within the STEC field is how to explain the paradox of the differential toxicity of the Stxs *in vitro* as compared to *in vivo*. We recently found that Stx2a is more toxic than Stx1a by the oral route in mice, data that supports the hypothesis that Stx2a is more potent from the gut and not just when injected intraperitoneally (99). One possible explanation for the differential toxicity of the toxins is that in contrast to epithelial cells such as Vero, the endothelial cells targeted in the HUS are more sensitive to Stx2a than Stx1a, and O’brig’s laboratory did find that renal microvascular endothelial cells obtained from human glomeruli are about 1000-fold more sensitive to Stx2a than Stx1a, although umbilical vein endothelial cells were equivalently sensitive (100). Since the Stxs are equally enzymatically active and they both bind Gb3, the question remains why different cell types exhibit differential sensitivity to the two Stx types. However, Gb3 does not exist in a homogeneous population within the cell, and it may be that the toxins do not bind equivalently to the Gb3 in target cells. An *in vitro* study with soluble forms of the trisaccharide moiety of Gb3 showed that Stx1a and Stx2a bind with similar affinity for that component of the receptor (64). Furthermore, Stx1a and Stx2a demonstrate similar affinities for renally-derived Gb3 as measured by enzyme-linked immunosorbent assay (ELISA), though by thin-layer chromatography Stx2a exhibits less binding than Stx1a (101). Examinations of toxin interaction with immobilized trisaccharides suggest that Stx1a has a higher affinity for the carbohydrate than does Stx2a (50, 102). Other experiments that examined the capacity of the toxins to bind Gb3 analogs showed that Stx1a and Stx2a do exhibit differential binding to those analogs *in vitro* (103), but whether similar analogs exist or act functionally as receptors *in vivo* has not been demonstrated.

Membrane cholesterol may also mask a portion of the potential Stx1a-binding Gb3 pool in Vero cell membranes (104) and adult renal glomeruli sections as mentioned previously (59), and incubation of Stx1a but not Stx2a with Gb3, cholesterol, and phosphatidylcholine partially neutralizes the cytotoxicity of Stx1a but not Stx2a (103). Stx2a, in contrast, is neutralized by human serum amyloid component P and lipopolysaccharide (LPS) from O107 or O117 *E. coli* (105, 106). The fact that the above compounds neutralize either Stx1a or Stx2a specifically, and presumably by preventing the toxins from binding to the cell, supports the hypothesis that the B pentamers of these toxins bind differently to cells. The Stx1a B pentamer is more stable than the comparable binding moiety of Stx2a (64) and that difference was shown to be at least partially due to a single aa residue within the B subunit (107). A study that examined the interaction of Stx1a and Stx2a with lipid rafts and subsequent intracellular trafficking in Vero cells showed that although both toxins associated with lipid rafts, Stx2a was also found in detergent-soluble (non-lipid raft) fractions of the membrane (108). In addition, some of the internalized Stx2a, but not Stx1a, co-localized with the cell surface marker transferrin, a finding that suggests that Stx2a is endocytosed from different areas of the membrane than Stx1a. However, both toxins localized to the Golgi after 1 h incubation with the Vero cells, although there was a suggestion that Stx2a exited from the Golgi at a slower rate than Stx1a.

At the level of the immune response to the toxins, Stx1a and Stx2a elicit differential chemokine responses from human umbilical vein endothelial cells (109) and a renal tubular epithelial cell line, HK-2 (110). The HK-2 cells are more sensitive to Stx1a than Stx2a and Stx2a-treatment induces expression of the macrophage chemoattractants macrophage inflammatory protein-1 α (MIP-1 α) and MIP-1 β (110). However, in a baboon intoxication model, there was no change in MIP-1 β in response to either Stx1a or Stx2a (111). Nonetheless, in that baboon model many characteristics of the HUS are observed, and Stx2a is lethal at lower doses than Stx1a, although the kidney injury takes longer to develop in the Stx2a-treated animals.

Taken together these studies on the differences between Stx1a and Stx2a indicate that the interaction between each toxin and the receptor is complex and influenced by several factors. Four distinctions between the Stx and Stx2a crystal structures that may contribute to the known biological differences exhibited by these toxins as discussed above are as follows: i) the Stx active site is partially blocked by the N-terminal region of the A₂ peptide; ii) The Stx2a A₂ C terminus has a more ordered structure than that of Stx; iii) Gb3-binding site 2 has a different conformation in each of the toxins; and, iv) Gb3-binding site 3 is partially blocked by the C-terminal two aa of the Stx2a A₂ peptide (28).

Stx ASSOCIATION WITH DISEASE

HUS cases were reported in the literature as early as the 1950s, though the cause was unknown. An understanding of the origins of infection-associated HUS was also complicated by the fact that some cases of the HUS are of genetic origin (see review (112)). However, in 1983 bloody diarrhea and the HUS were linked to certain serogroups of *E. coli* and O'Brien et al. found that those strains produced a toxin related to the Stx of *S. dysenteriae* (3, 4, 113). The strong association between the HUS and Stx2a was underscored in the largest outbreak of the HUS in Germany in 2011 in which more than 800 cases of the disease were identified (114). The surprise from German outbreak was that although the implicated strain was an *E. coli*, unlike typical STEC, the epidemic isolate also encoded virulence factors associated with enteroaggregative *E. coli* (EAEC). EAEC had only rarely been associated with the HUS previously, and in each of those cases the implicated isolate was found to carry an Stx gene.

In animal models, purified Stx in the absence of detectable LPS is linked to kidney damage and death (99, 115). *S. dysenteriae* mutants that lack *stx* caused a milder dysentery in monkeys and produced only a trace of blood in stool compared to the wild-type isolate (116). Furthermore, Stx can be detected in the kidneys of some patients (117–119). Animal models further show that antibodies to the Stxs are protective, and humanized versions of those antibodies have completed Phase I trials (120, 121).

STEC Pathogenesis

STEC pathogenesis requires ingestion of the bacteria in contaminated food or water, though the organism may occasionally be passed person-to-person. To cause disease the organism colonizes the intestine, a process that likely includes attachment as well as proliferation. That STEC that carry the locus of enterocyte effacement (LEE) use the adhesin intimin

(encoded by the *eae* gene) to adhere within the intestine has been shown in mouse and pig models (122, 123). In addition, STEC that are *eae* positive are more likely to cause disease than intimin-negative strains even in non-O157 strains (8, 124). We recently demonstrated in mice that a high fiber diet may influence the development of disease after O157 infection (74, 125). The influence of the higher fiber diet was two-fold and was associated with the production of butyrate within the intestine: mice on a high fiber diet were colonized to a greater degree than mice on a low fiber diet perhaps as a consequence of reduced overall levels of competitive native *Escherichia* species, and increased levels of Gb3 were observed in both the intestine and kidney.

After colonization with STEC, the innate proinflammatory response may initially be suppressed in those infected with strains that are LEE+ (see review (90)), a conclusion supported by the fact that STEC infection is generally not an inflammatory diarrhea. In the intestine the STEC elaborate Stx(s), and the patient may develop hemorrhagic colitis, the pathogenesis of which is not clearly understood. For systemic consequences, the Stx(s) enter the host from the intestine and reach target endothelium cells in the kidney and in some cases, the central nervous system. The Stx(s) damage cells directly and cause apoptosis as described above. The Stx(s) and likely LPS also elicit cytokines and other immune mediators within the kidney and from monocytes (for a review see (90)). Endothelial cells within the glomeruli become damaged and may die and detach from the membrane. Extrusion of the cell exposes collagen, normally not present within the blood vessel, and platelets become activated. The presence of activated platelets encourages fibrin deposition and leads to the development of a thrombus and promotes a coagulation cascade. Recent studies suggest that Stx-treatment of endothelial cells can induce the expression of factors that may stabilize clot formation even in the absence of cell death or extrusion (see reviews (126, 127)). Such damaged endothelial cells also become a site for the adhesion of leukocytes, the consequence of which is additional damage to the kidney (128). The localized prothrombotic environment in the glomerulus leads to the reduction of platelets in the serum (thrombocytopenia), hemolytic anemia, and renal damage, the triad of the HUS (for reviews see (126, 129, 130)). Altered complement levels have been found in patients with STEC HUS (131, 132), but whether those changes are part of the etiology of the disease or occur as a consequence is not clear (see (127) for review of possible role for complement in STEC HUS).

SUMMARY

The Stxs are potent poisons associated with bloody diarrhea and the potentially severe disease, the HUS. The Stx(s) act as ribotoxins that halt protein synthesis within the cell and induce apoptosis, but can also prompt altered gene/protein expression in epithelial cells, endothelial cells, monocytes, and mesangial cells. The consequence of exposure to *E. coli* that elaborate one or more Stx can range from asymptomatic infection to bloody diarrhea and, in some patients, the HUS. In the research arena, the Stxs have been exploited beautifully to interrogate mechanisms of retrograde transport, and to localize sites of Gb3 expression, and, more recently, the possible function of tubular invaginations in membranes.

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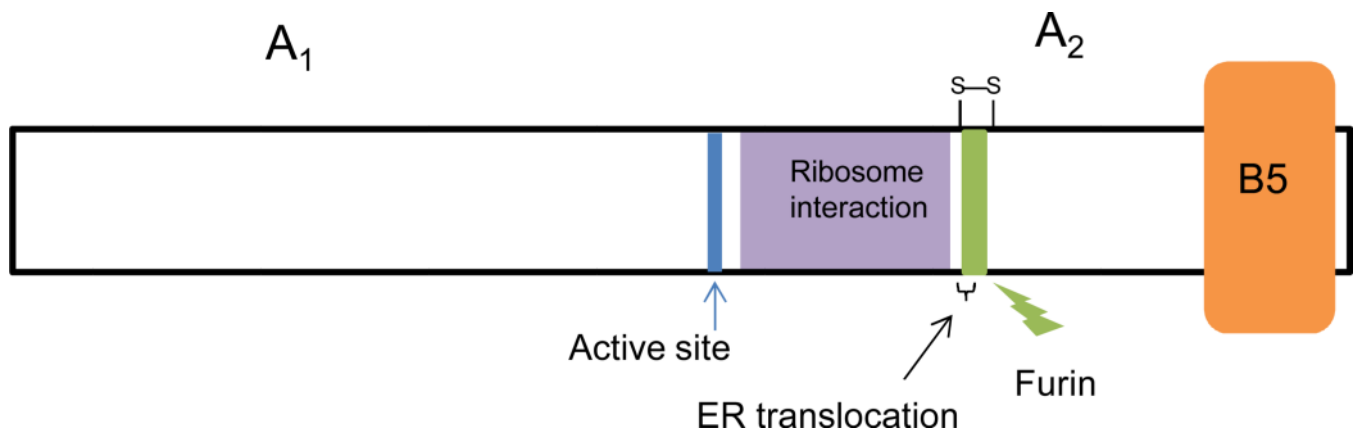


Figure 1. Cartoon representation of the Stx structure

The active site glutamic acid is indicated as a vertical blue line, the ribosome interaction region is shown in purple, the protease (Furin) sensitive site is depicted in green, and the B pentamer as an orange block. The disulfide bridge that connects the A₁ subunit and the A₂ peptide is shown above the protease sensitive site. A region important for translocation from the ER to the cytosol is indicated by a bracket. Not to scale.

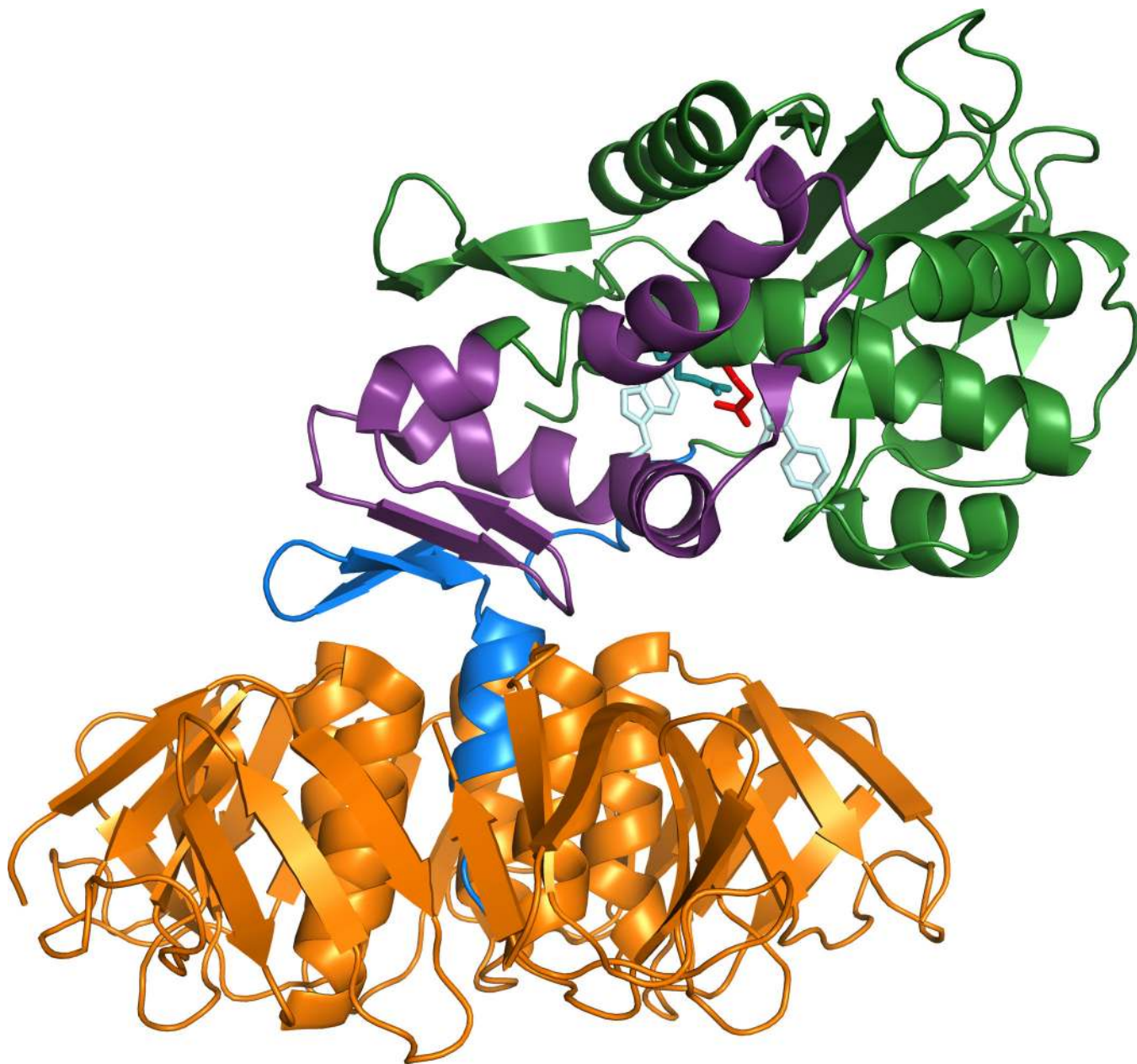


Figure 2. Ribbon diagram of the Stx1 crystal structure

The B pentamer is shown in orange and the A₂ in blue. The majority of the A₁ is depicted in green except for the region that interacts with the ribosome, which is shown in purple. The active residue 167 is red and other active-site side chains are pale blue. The A₂ chain is medium blue and the B subunits are orange. The structure (1R4Q) was drawn with PyMOL Molecular Graphics System, Version 1.5.0 Schrödinger, LLC. Figure kindly provided by Dr. James Vergis.

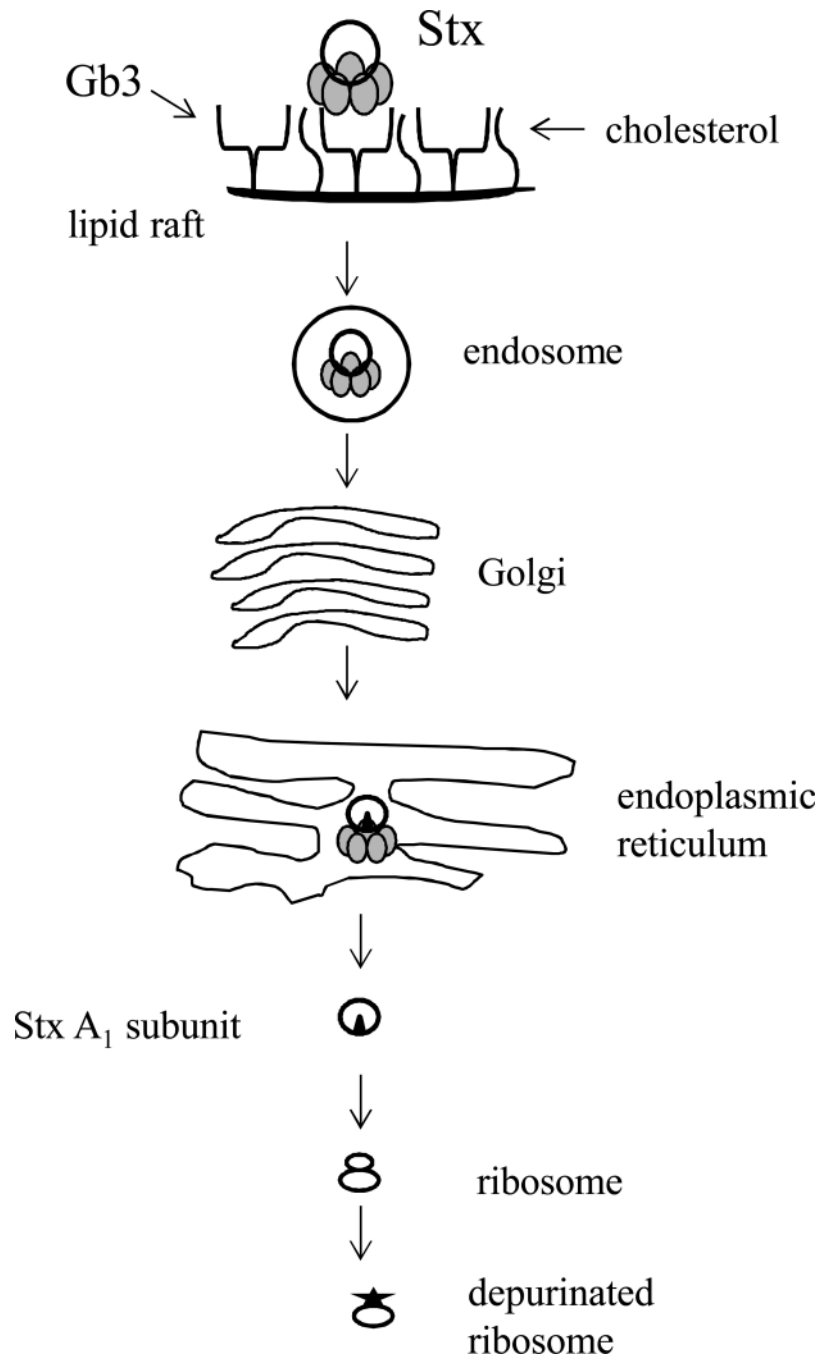


Figure 3. An illustration of the retrograde pathway for Stxs

The toxin binds to Gb3 within lipid rafts that contain cholesterol and that complex is internalized within an endosome. From the endosome the toxin traffics to the Golgi where it is nicked by furin if that nicking did not occur in the intestine. The nicked toxin moves to the ER where the disulfide bridge that keeps the A₁ tethered to A₂B₅ is reduced. The A₁ chain then enters the cytosol and removes an adenine residue from the 28S ribosome.

Table 1

Prototype toxins and strains that produce those toxins

Toxin type(s)	Prototype strain used for determination of <i>stx</i> subtype ^a	Linked with serious human disease; difference(s) from prototype toxin ^b	Reference(s)
Stx	3818T	Yes	(133)
Stx1a	EDL933 (makes Stx1a and Stx2a)	Yes	(113)
Stx1c	DG131/3	No; immunologically distinct	(134, 135)
Stx1d	MHI813	No; immunologically distinct, less potent	(136)
Stx2a	EDL933 (makes Stx1a and Stx2a)	Yes	(113)
Stx2b (originally named VT-2d or Stx2d)	EH250	No; the B subunit gene was not detected by methods used to detect other <i>stx</i> B subunit genes	(24)
Stx2c	031	Yes, less toxic to Vero cells and mice	(137)
Stx2d (Stx2dact)	C165-02	Yes; more toxic after incubation with elastase, less toxic to Vero cells	(138)
Stx2e	S1191	No; binds to Gb4, associated with disease in pigs	(139)
Stx2f	T4/97	No; originally isolated in STEC from pigeons; immunologically distinct	(140)
Stx2g	7v	No; the <i>stx</i> _{2g} gene is not amplified by primers specific for <i>stx</i> _{2a}	(141)

^aMore information about the prototypes strains may be found in reference (10).

^bPrototype toxin indicated in bold.