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Molecular aspects of effect of Shiga toxin in humans – A Review

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ABSTRACT

Shiga toxins belonging to a family of structurally and functionally related protein toxins serve as the key virulence factors for pathogenecity of the virulent enteric bacterial strains namely *Shigella dysenteriae* serotype 1 including Shiga toxin-producing *Escherichia coli* (STEC). *S. dysenteriae* type 1 isolates remain major public health concerns due to their widespread outbreaks and the severity of extra-intestinal diseases, including acute renal failure and also affects the central nervous system. Despite practicing improved hygienic conditions and regulating food and drinking water safety, the enteric pathogens are imposing a major threat in the well being of the human society. Shiga toxin on entry into host cells' endoplasmic reticulum (ER) activates the stress response in ER and inhibits protein synthesis by catalytic inactivation of eukaryotic ribosomes. In many cell types shiga toxins trigger apoptosis. Recent studies have shown that shiga toxins induce autophagy which activates different signaling pathways in toxin-sensitive and toxin-resistant human cells. In this review the molecular basis of Shiga toxins' effect on host cell leading to manifestation of the infection in affected individuals are discussed with an emphasis on recent findings.

Keywords: Shiga toxins; STEC; Apoptosis; *Shigella dysenteraie*; Infection

1. INTRODUCTION

Shiga toxins (Stxs) are genetically and structurally related cytotoxins expressed by the enteric pathogens *Shigella dysenteriae* serotype 1 and number of Shiga toxin producing *Escherichia coli* (STEC) serotypes (Gyles, 2007). Shiga toxin (Stx) is a virulence factor also produced by Enterohemorrhagic *E. coli* (EHEC) (Karmali et al., 1985). It is also known as Verotoxin (VT) due to Vero cells' sensitivity to Stxs (Konowalchuk et al., 1977). STX is the member of the toxin family, which is expressed by *S. dysenteriae* serotype 1. The Stxs expressed by STEC may be categorized as Shiga toxin type 1 (Stx1), which is essentially identical to Shiga toxin, and Shiga toxin type 2 (Stx2), which is 56% homologous to Shiga toxin/Stx1 as revealed by amino acid sequencing (Jackson et al., 1987). Stxs are encoded by late genes of lambdoid bacteriophage (Allison, 2007). Stxs show a typical AB5 molecular structure, composed of one enzymatically active A subunit (32 kDa) and five identical B subunits (7.7 kDa each) that mediate the attachment of holotoxin (AB5) to neutral glycolipid

receptor globotriaosylceramide (Gb3, or CD77) on the surface of host cells (Stein et al., 1992; Fraser et al., 1994). StxA and StxB subunits are separately secreted into the bacterial periplasm where they are noncovalently assembled into holotoxin (Donohue-Rolfe and Keusch 1983).

Stx is an essential virulence factor in human disease mediated by *E. coli* O157:H7. This strain was responsible for the first documented outbreak of haemorrhagic colitis in the US in 1982 (Riley et al., 1983).The two antigenically distinct Stx variants (currently known as Stx1 and Stx2) produced by *E. coli* are related to the Shiga toxin produced by *S. dysenteriae* (O'Brien et al., 1982; Scotland et al., 1985; Strockbine et al., 1986; Strockbine et al., 1988), which was first identified over 100 years ago (Bridgwater et al., 1955). The similarity is particularly high (one amino acid difference) between the sequences of *E. coli*-derived and the Stx of *S. dysenteriae*. In addition to *Shigella and E. coli*, stx variants have been shown to be expressed by *Enterobacter*, *Citrobacter*, *Acineobacter*, *Campylobacter*, and *Hamiltonella* bacterial species (Moore et al., 1988; Schmidt et al., 1993; Haque et al., 1996; Paton and Paton 1996; Grotiuz et al., 2006; Degnan and Moran 2008; Alperi and Figueras 2010). Thus, the genes encoding Shiga toxin may be broadly distributed among bacterial isolates.

Stxs are essential for the development of severe bloody diarrhea and hemolytic- uremic syndrome (HUS) characterized by serious complications including renal failure, thrombocytopenia and hemolytic anemia (Karmali et al., 1983). Internalization of Stx into the target cell by endocytosis lead to transportation via the trans- Golgi network to the endoplasmic reticulum (ER) and finally into the cytoplasm (Sandvig et al., 1992). The elongation-factor-1-dependent aminoacyl tRNA binding is blocked by depurination reaction, thereby rendering ribosomes inactive for protein synthesis which leads to cell death in susceptible host cells (Iordanov et al., 1997). This effect is termed as the ribotoxic stress response (Iordanov et al., 1997). Stx also triggers programmed cell death (apoptosis) through various mechanisms in different cell types (Cherla et al., 2003). Thus, recent studies have focused on the exploration of cell death signaling mechanisms activated by the toxins. The purpose of the present review is to assess the effects of shiga toxin on human health in the present scenario.

2. SHIGA TOXIN

Shiga toxins (Stxs) are type of cytotoxins which are genetically and structurally related and expressed by the enteric pathogens *S. dysenteriae* serotype 1 and an expanding number of Shiga toxin producing *E. coli* (STEC) serotypes (Gyles, 2007). Ingestion of small numbers of Stx-producing bacteria in contaminated food or water may lead to bloody diarrhea (bacillary dysentery or hemorrhagic colitis). Unfortunately, these patients are at risk for developing life-threatening extra-intestinal complications including acute renal failure and neurological abnormalities such as seizures and paralysis (Tarr et al., 2005; Proulx and Tesh, 2007; Obata, 2010). Stxs are a type of AB5 holotoxins, consisting of an enzymatic A-subunit (~32-kDa) in non-covalent association with five B-subunits, each B-subunit protein being ~7.7 kDa. B-subunits pentamerize to form a ring and the C-terminus of the A-subunit inserts into the central pore (Fraser et al., 2004). B-subunits of Stxs that cause disease in humans bind to the neutral globo-series glycolipid globotriaosylceramide (Gb3). Following toxin binding to Gb3-expressing cells, the holotoxin is internalized initially involving the B subunit-induced negative curvature of the host cell membrane leading to the formation of toxin-containing membrane invaginations (Römer et al., 2007). The toxin reaches the ER by undergoing a

process termed as the retrograde transport involving the endosomes to the *trans*-Golgi network, through the Golgi apparatus. During transport, the A-subunit is cleaved by furin or a furin-like protease to form a 27 kDa A1- fragment and a 4 kDa A2-fragment which remain associated via a disulfide bond (Sandvig et al., 1992; Garred et al., 1995). The ER is the site of disulfide bond reduction, unfolding of the A1-fragment and retrotranslocation of the A1-fragment possibly via the Sec61 translocon into the cytoplasm (Lord et al., 2005; Tam and Lingwood, 2007). The A1- fragment possesses *N*-glycosidase activity and cleaves a single adenine residue from the 28S rRNA component of eukaryotic ribosomes (Endo et al., 1988).

The depurination target is called the GAGA tetraloop. This region of the 28S rRNA is also called the α-sarcin/ricin loop since the enzymatic action of these two ribosome-inactivating proteins (RIPs) is also directed to this loop. The depurination reaction blocks association with elongation factors, resulting in protein synthesis inhibition (Vernon, 2012). Since the Vero cells (African green monkey renal epithelial cells) are highly sensitive to protein synthesis inhibition and cell death by Stxs, the toxins are also referred to as verotoxins or verocytotoxins (Johannes and Römer, 2010; Lingwood et al., 2010; Sandvig et al., 2010). While the enzymatic action of Stxs is well characterized, the precise relationship between rRNA depurination/protein synthesis inhibition and cell death remains unclear. It has become evident that Stxs induce apoptosis or programmed cell death in many cell types *in vitro* and *in vivo* (Tesh, 2010). Thus, recent studies have focused on the exploration of cell death signaling mechanisms activated by the toxins. Stxs are effective signaling molecules activating multiple stress responses in eukaryotic cells. While protein synthesis inhibition may lead to cell death, Stx-induced protein synthesis inhibition may be dissociated from cell death signaling in some cell types. Recent studies examine cell stress responses activated by Stxs following the depurination reaction (ribotoxic stress response) or by the presence of unfolded proteins within the ER (unfolded protein response). Signaling through these pathways may be involved in the induction of cytokine/chemokine expression and programmed cell death, processes which contribute to the pathogenesis of disease caused by Stxs (Vernon, 2012).

2.1. Origin of Shiga Toxin

The genes for Stx in *S. dysenteriae* are located on the chromosome (O'Brien et al., 1992). However, the Stx genes in *E. coli* originated from cryptic lambdoid prophages. The enterohemorrhagic *E. coli* (EHEC) strain (*E. coli* O157:H7 strain 933) that caused the first US outbreak of haemorrhagic colitis (Riley et al., 1983) encodes both Stx1 and Stx2. Genes encoding these toxins in this strain are located on different lysogenic lambdoid bacteriophages. Bacteriophage that separately encode Stx1 and Stx2 have been isolated from various EHEC strains, and these phage are now representative of the Stx-encoding prophages. Since original isolation of Stx1 and Stx2 lambdoid bacteriophage from Shiga toxin encoding *E. coli* (STEC), many other Stx-encoding phages have been isolated and their sequences at least partially characterized. These data confirm that the Stx2-encoding phage have the general lambda-like organization of genes. The overall construction of Stx1 phages is similar to that of Stx2-encoding phages, and their genes display some similarity (Sato et al., 2003), whereas other Stx1 phages contain different sets of homologous genes. The component genes of individual lambdoid phages are arranged modularly and are generally a mosaic construction of genes from a particular family of genes. This modular and mosaic structure extends to individual domains of single proteins. For example, the sequence of the DNA binding domain at the *N*-terminus of the repressor of phage 933W is nearly identical to that of the non-toxic lambdoid phage HK022, but the sequence of the *C*-terminal oligomerization

domain of this protein is identical to that found in the repressor of the Stx1-encoding phage H19-B (Fattah et al., 2000).

The opportunity to create new species via recombination is shown by analysis of the genomic sequence of the *E. coli* O157:H7 strain EDL933 genome (Perna et al., 2001). This strain harbors two active bacteriophage, each expressing either *stx1* or *stx2* genes. This finding demonstrates that a STEC strain can harbor more than one Shiga toxin encoding bacteriophage. In addition, this strain is lysogenic for many cryptic phage genomes, providing ample material for recombination and a strategy for rampant spread of Stx in the environment (Mauro and Koudelka, 2011).

2.2. Sources of Shiga Toxin Infection in Humans

A PCR-based approach of bacterially enriched bovine fecal samples gave consistent results of *stx* gene contamination (Rogerie et al., 2001; Jenkins et al., 2002; Gilbreath et al., 2009; Menrath et al., 2010). This finding is corroborated in the earlier studies which that in terrestrial environments cows act as hosts for Stx producing bacteria that have been implicated in human illness through fecal contamination of beef, plants, and soils in the nearby environment (Caprioli et al., 2005; Hussein, 2007; Fremaux et al., 2008; Ferens and Hovde, 2011). In another study 89% of different cattle fecal samples tested positive for *stx* when phages were examined by qPCR (Imamovic et al., 2010). Moreover, in a study that analyzed the presence of *stx* genes in dairy cows and calves in Argentina, a detection frequency of *stx* in the cow feces sampled ranged from 4% to 60%, a variance that was dependent on the season in which the feces were collected (Fernandez et al., 2009). Regardless of the variability of Stx detection in different studies, it is clear that cows are a major reservoir for Stx-producing microbes. In one study, over 100 fecal samples obtained from sheep and lambs were analyzed, and it was found that 87.6% of the samples tested positive for the *stx* gene (Urdahl et al., 2003). Thus it is becoming evident that other agriculturally based animals must now be considered in this matter. Analysis of *E. coli* isolates obtained from sheep feces in another study found that 65.9% of the sheep tested positive for microbes carrying the *stx* gene (Cookson et al., 2006). Furthermore, *E. coli* O157 collected from sheep feces in a Netherland slaughterhouse were shown to both contain *stx* genes and to be cytotoxic to Vero cells, suggesting that these strains are able to produce Stx protein (Heuvelink et al., 1998). This report indicates that sheep are similar to cows in their ability to harbor Stx-producing organisms.

An analysis of *E. coli* strains isolated from goat feces provides a range between 10%–50% of goats that contain microorganisms which possess a *stx* gene, some of which have been shown to be toxic to Vero cells (Corte's et al., 2005; Wani et al., 2006; Vu-Khac et al., 2008). Similar levels of *stx* genes and cytotoxic potential of STEC has been established in pigs and buffalos (Galiero et al., 2005; Oliveira et al., 2007; Fratamico et al., 2008; Kim et al., 2010; Yan et al., 2011).

Recent studies have shown that other animals, besides those involved in agriculture, harbor Stx producing bacterial isolates. Over 50 STEC strains have been isolated from deer feces and shown to contain *stx* genes and exhibit Vero cell cytotoxicity (Asakura et al., 1998; Sánchez et al., 2009). Survey of 50 fecal samples obtained from white tailed deer in Pennsylvania, it was found that nearly 50% of the samples contained a *stx* gene. Studies performed among other animals such as dogs, cats, wild boars, and various zoo animals; including yaks, alpacas, antelopes, and llamas have also provided evidences as hosts for Stx-producing microorganisms (Leotta et al., 2006; Bentancor et al., 2007; Sánchez et al., 2010) Wild bird

populations have also shown to harbor organisms which possess the *stx* gene (Hughes et al., 2009). Additionally, airborne particulates in a contaminated building have been shown to contain bacteria with *stx* genes that have been linked to an outbreak of STEC in humans (Varma et al., 2003). Thus from various studies it is quiet clear Stx-producing organisms are highly abundant in a variety of terrestrial ecosystems and can act as good sources for human infection.

The aquatic environments have also been shown to harbor Stx-producing organisms that have been linked to a number of STEC outbreaks (Muniesa et al., 2006). For example, Stx has been implicated as a possible pathogenic agent in drinking water responsible for gastrointestinal illness outbreaks (Swerdlow et al., 1992; Licence et al., 2001; Yatsuyanagi et al., 2002; Bopp et al., 2003; McCall et al., 2010; Lienemann et al, 2011). In India one study reported the presence of *stx* genes in drinking water containing 30% contamination of *E. coli* isolates harboring either *stx1* or *stx2* (Ram et al., 2008). In a larger study conducted in Austria, over 200 *E. coli* isolates were obtained from various drinking water sources, and only one was found to contain *stx2* (Halabi et al.,2008). Water used for recreational purposes has also been found to contain organisms which produce Stx and cause human illness (McCarthy et al., 2001; Paunio et al., 1999; Feldman et al., 2002). In a similar analysis in beach waters of Lake Erie, over 10% of the samples tested positive for the *stx2* gene (Smith et al., 2009). When fecal coliform isolates obtained from water samples on the California coast line was probed for *stx* DNA, a similar frequency of detection was observed in comparison to the Lake Erie beach waters study (Walters et al., 2011).

Stx gene containing bacterial isolates were obtained from river Ganga water samples where 22.7% of *E. coli* isolates were obtained. More than 50% of fecal coliform isolates tested positive for *stx2* DNA in river water in Maryland and river water samples obtained in Michigan and Indiana (Ram et al., 2007; Duris et al., 2009). The major cause for the appearances of Stx producing bacterial water in natural water bodies is likely to be the influx of non-point sources pollution, such as faecal contaminated wastewater (Muniesa et al., 2006). The frequency of *stx* positive *E. coli* has been shown to be even higher in non-sewage wastewaters. For example, in an analysis of *E. coli* isolates from 224 wastewater samples obtained from slaughterhouses in France, it was found that 13% tested positive for the *stx2* gene (Loukiadis et al., 2006). Presence of bacteriophages in wastewater which contain *stx* genes have also been documented several studies. For example, 70% of urban sewage samples and 94% of animal wastewater samples tested positive for phages that contain the *stx2* gene, which could reach values of up to 1010 gene copies/mL of wastewater (Imamovic et al., 2010; Muniesa and Jofre, 2000). These studies demonstrate that both bacteria and bacteriophages which contain *stx* genes are abundant in wastewater. The contaminated untreated drinking water as well as the recreational water sources is the potent reservoirs for Stx producing bacterial strains that is responsible for human infection.

3. PATHOGENECITY OF SHIGA TOXIN IN HUMANS

Enterohemorrhagic *E. coli* (EHEC) are a sub-group of STEC that have the ability to form attaching and effacing lesions (A/E lesions) that facilitate intimate adhesion to the intestinal mucosa (Boerlin et al., 1999). Stx-producing *E. coli* O157:H7 has become recognized as the leading cause of HUS worldwide (Brzuszkiewicz et al., 2011). The principal route of infection is consumption of contaminated food and drinking water, although infection may also occur via direct contact with animals, person-to-person contact, and

environmental exposure (Buchholz et al., 2011; Carey et al., 2008). After ingestion of the EHEC pathogen, its adhesion and virulence factors are activated and lead to colonization of the terminal ileum and the epithelium of the Peyer's patches. Thereafter, several virulence factors (e.g., Stx and LPS) are released from the bacterium (Phillips et al., 2000). As bacteremia is uncommon during HUS, it is likely that only the toxin is transported to the site of pathological lesions (the glomerular endothelium, brain, and/or pancreas). Stx released by decaying bacteria in the gut migrates through the intestinal barrier, binds to platelets in the blood, and is transported to the target organs. Stxs interact via subunit B with their cellular receptor (Gb3 [globotriaosylceramide]). Gb3 facilitates the endocytosis and intracellular trafficking of the toxin. Within the host cell, the Stx A-subunit cleaves the rRNA at a specific position (O'Brien et al., 1992), and this leads to the inactivation of the protein machinery and results in cell death. Since renal glomerular capillary thrombotic microangiopathy is the characteristic of EHEC-associated HUS, the cytotoxicity of Stx is probably directly linked to the pathogenesis of HUS (Kaplan et al., 1998). Several studies have verified the suspected role of the Stx B subunit for the initiation of the apoptotic pathway (Nakagawa et al., 1999). Several virulence genes promoting intestinal colonization of EHEC are located on pathogenicity islands, but these loci were not found in the epidemic O104:H4 strains (Bielaszewska et al., 2011; Mellmann et al., 2011).

A leading cause of acute renal failure in the pediatric population is most prevalent in infants and young children is associated with STEC induced HUS (Scheiring et al., 2008). Histopathological manifestations of Stx-associated HUS include fibrin-rich microvascular thrombi primarily in the renal glomeruli, although pre-glomerular arterioles and medium-sized vessels may also be affected (Petruzziello-Pellegrini and Marsden, 2012). Endothelial swelling and detachment from the underlying basement membrane and sub-endothelial deposits accompanied by vascular edema and narrowing of the vessel lumen are also observed (Tarr et al., 2005; Jansen and Kielstein, 2011). Although the kidneys are the prime targets, extra-renal complications may develop. Moreover neurological complications occur in 10% to 25% of patients and is the most common cause of mortality during the acute phase of disease (Tarr et al., 2005; Trachtman et al., 2012). In certain cases cardiac complications are associated with a high risk of mortality in HUS patients (Siegler, 1994). It is reported that about 12% risk of mortality is associated with the end-stage renal disease with STEC-HUS is and 25% of survivors experience long-term chronic kidney diseases including decreased glomerular filtration rate, proteinuria and hypertension (Zoja et al., 2010; Rosales et al., 2012). Antibiotic treatment is generally discouraged because of the potential to enhance the synthesis and release of Stx from EHEC and worsen disease severity Recent investigations suggest that the antibiotic ciprofloxacin increases Stx2 production in case of *E. coli* O104:H4 infection, while other antibiotics either suppress or do not affect toxin expression (Bielaszewska et al., 2012).

Several groups have studied the effects of Stx on vascular endothelial cells in an effort to understand the endothelial dysfunction mediated by Stx. Louise and Obrig have shown that Stx induces an increase in the ratio of plasminogen activator inhibitor-1 (PAI-1) to tissue plasminogen activator (tPA), suggesting that toxin treatment induced an endothelial phenotype favoring stabilization of fibrin clots (Louise and Obrig, 1994). Recently, Stx was shown to upregulate the chemokine stromal cell-derived factor-1 (SDF-1) in cultured endothelial cells and in children with STEC-HUS (Petruzziello-Pellegrini et al., 2012).

4. DISCUSSION AND CONCLUSION

Bacterial genome sequences analyses reveal that the genomes of both cryptic and active temperate phage are found with surprisingly high frequency in the chromosomes of these organisms. Although these toxins do affect humans and other mammals, these phage-encoded exotoxin genes are found at high frequencies in free phages and lysogenic bacteria isolated from environments where the presumed corresponding targets are not prevalent (Casas et al., 2006). The prevalence of phage DNA, especially phages encoding exotoxins, inside host chromosomes suggests that these toxin-carrying phages are well tolerated in the bacterial genomes because their presence provides an evolutionary advantage to the host. Phage-borne exotoxins like Stx provide a bacterial population with the ability to combat predation and provide an explanation for the prevalence of phage-encoded exotoxin genes in the biosphere. These observations have led to the fact that humans and other susceptible mammals are neither the original nor primary targets of these toxins (Matz and Kjelleberg, 2005). Stxs are critical factors for the development of diseases such as severe bloody diarrhea and hemolytic uremic syndrome. Additionally, Stxs trigger the secretion of pro- inflammatory cytokines and chemokines, particularly in monocytes or macrophages. The inflammatory cytokines result in the modulation of the immune system, local inflammations and enhancement of cytotoxicity (Moazzezy et al., 2014). Shiga toxin-producing *E. coli* represents a significant global health concern, especially as hypervirulent pathogens surface amidst outbreaks of hemolytic uremic syndrome (HUS). Stx is key regulator in the microangiopathic events underlying the disease. The mechanisms of Stx mediated endothelial dysfunction have been a major focus of research that has provided new insights into the pathogenic changes in endothelial structure leading to HUS. Among the newer concepts are Stx-mediated gene regulation in the absence of protein synthesis inhibition, a potential role for complement activation, and accumulating evidence for detectable serum markers before the onset of the classic clinical features of HUS. Further investigation of newer therapeutic targets and potential prognostic markers is essential to assess their utility in mitigating disease and/or predicting outcomes and will provide an improved overall understanding of Stx-induced HUS pathogenesis (Petruzziello-Pellegrini et al., 2013). Till date little information is available on the regulation of Stx expression and toxin transport in the human gut. Further research is necessary to address the influence of environmental factors in the human intestine on Stx production and release. A better understanding of Stx-related events in the human gut is necessary since as it will lead to the development of early detection and treatment strategies against HUS (Schüller et al., 2011). In the present review it is concluded that current knowledge about the molecular structure of Stxs, biochemical aspects of Stxs effect on human health and the sources of this potent enterotoxin are well documented in the present era of research. The purpose of this review is to focus on the fact that future research should address more on the influence of the intestinal environment on Stx production and release, Stx interaction with intestinal epithelial cells and intracellular uptake and toxin translocation into underlying tissues to cope up with the changing trend of this Stx bearing pathogenic bacterial strains which are imposing threats in the survival of mankind.

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