REVIEW

Diarrhea induced by infection of *Vibrio parahaemolyticus*

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Abstract : *Vibrio parahaemolyticus* is a human pathogen that naturally inhabits marine and estuarine environments. Infection with *V. parahaemolyticus* is often associated with the consumption of raw or undercooked seafood, causing gastroenteritis with watery diarrhea. The presence of two type III secretion system (T3SS) proteins, thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), has been closely associated with the severity of diarrheal illness. TDH and TRH have various biological activities including hemolytic activity, cardiotoxicity, and enterotoxicity. T3SS1 is involved in cytotoxicity to host cells and orchestrates a multifaceted host cell infection by induction of autophagy, cell rounding, and cell lysis. T3SS2 is thought to be related to the enterotoxicity of *V. parahaemolyticus*. The activities of inducing diarrhea of each of the virulence factors were summarized in this review. J. Med. Invest. 57 : 179-182, August, 2010

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Many species of bacteria induce secretory and inflammatory diarrhea (1). The increased intestinal fluid secretion in diarrhea appears to result from the active secretion of chloride (1-3), a principal anion, but the way in which diarrhea is caused by individual bacterial infections has not been completely elucidated. *Vibrio parahaemolyticus* is a Gram-negative halophilic bacterium that naturally occurs in marine and estuarine environments (4). It is a human pathogen that causes food-borne acute gastroenteritis, often associated with the consumption of raw or undercooked seafood (5, 6). Clinical symptoms of *V. parahaemolyticus* infections include watery diarrhea, abdominal cramps, nausea, vomiting, headaches, fever, and chills (7, 8).

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THERMOSTABLE DIRECT HEMOLYSIN (TDH) AND TDH-RELATED HEMOLYSIN (TRH)

The majority of *V. parahaemolyticus* clinical isolates from patients with diarrhea has produced TDH and/or TRH, which are encoded by the *tdh* and *trh* genes, respectively (9). Strong associations have been found between gastroenteritis and these two proteins (10, 11). Therefore, TDH and TRH are regarded as major virulence factors of *V. parahaemolyticus*.

V. parahaemolyticus TDH (Vp-TDH) is a proteinaceous toxin composed of 165 amino acids with one disulfide bond near the carboxyl terminus (12). The protein is a dimer, which lacks lipid and carbohydrate moieties, and has a molecular weight of *c.* 42 kDa by gel filtration and 21 kDa by denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (13, 14). Vp-TDH exhibits β-hemolytic activity on Wagatsuma medium, which has been termed the Kanagawa phenomenon (KP). Purified Vp-TDH is heat-stable, even at 100°C for

10 min (14, 15) and has hemolytic, cytotoxic, enterotoxic, mouse lethality and cardiotoxic activities (14). It is believed that Vp-TDH damages the erythrocyte membrane by acting as a pore-forming toxin, with the pores estimated at 2 nm in diameter (Vp-TDH also has the ability to lyse target eukaryotic cells by punching holes in the plasma membrane) (16).

Evidence suggests that Vp-TDH-induced hemolysis occurs in three sequential steps : 1) binding to the erythrocyte membrane, 2) formation of a transmembrane pore, and 3)disruption of the cell membrane (16). The N-terminal region is thought to be involved in the binding process, whereas the region near the C-terminal region has been implicated in post-binding activities (17). It is clear that phosphorylation of a 25-kDa host protein induced by Vp-TDH is essential for hemolysis after binding to the erythrocyte membrane (18). In addition, Vp-TDH induces cation permeability and activates endogenous Gardos potassium (K^+) channels (19). The consequences of this activity include breakdown of phosphatidylserine asymmetry, which depends, at least partially, on cellular loss of K^+ (19).

The primary target of TDH appears to be intestinal epithelial cells. Thus, TDH effects on epithelial cells are important for biological functions, such as diarrhea. The addition of TDH to the mucosal side of human colonic tissue in Ussing chambers led to increased short circuit currents (Isc), a process that was inhibited by 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS), an inhibitor of Ca2+-activated chloride (Cl^-) channels. High Isc and intracellular Ca^{2+} concentrations ($[Ca^{2+}]_{in}$) were detected in human colonic epithelial (Caco-2) cells following the addition of TDH to the apical side of the cell monolayer. The Isc decreased with the addition of DIDS, but not with glybenclamide, 5-nitro-2-(3-phenylpropylamino) benzoic acid, or gadolinium chloride. An increase in Isc was not observed when the Cl^- in the medium was replaced by gluconate or when Ca^{2+} was depleted. Similarly, TDH did not raise $[Ca^{2+}]_{in}$ after depletion of extracellular Ca^{2+}. R7, a mutant form of TDH, reduced the effects of TDH on Isc and $[Ca^{2+}]_{in}$, as did protein kinase C (PKC) inhibitors. Thus, TDH increases Cl^- secretion in human colonic epithelial cells, apparently through mechanisms involving cell binding and Ca^2+ influx, followed by elevation of $[Ca^{2+}]_{in}$ associated with PKC phosphorylation (20).

Honda et al. reported that KP-negative isolates of clinical origin produce a TDH-related hemolysin, coined Vp-TRH, which is also regarded as an important virulence factor. Also a hemolytic toxin, TRH is produced by Kanagawa-phenomenon-negative *V. parahaemolyticus* and is suspected of playing an important, but yet-to-be-determined role in the diarrhea caused by this organism. In particular, Vp-TRH stimulates fluid secretion in the rabbit ileal loop test, which suggests a possible role for the toxin in inducing diarrhea and has an amino acid sequence that is approximately 67% homologous with Vp-TDH (21). However, unlike the *tdh* genes, significant nucleotide differences exist within the *trh* family, with two subgroups, *trh1* and *trh2*, sharing 84% sequence identity (22). Vp-TRH is also immunologically related to TDH and is heat labile at 60°C for 10 min (20). Both Vp-TDH and Vp-TRH induce chloride secretion in human colonic epithelial cells (20, 23). In cultured human colonic epithelial cells, TRH increases Cl(-) secretion, followed by elevation of intracellular calcium.

TYPE III SECRETION SYSTEM

Park et al. have shown that a *tdh* deletion mutant retains the ability to cause fluid accumulation (24). Furthermore, Lynch et al. (2005) reported that both *V. parahaemolyticus* TDH-positive and -negative strains are still disrupted in epithelial tight junctions (25). Those studies indicate that there are factors, in addition to TDH or TRH, that contribute to the pathogenesis of *V. parahaemolyticus*.

Analysis of the genome sequence of *V. parahaemolyticus* strain RIMD2210633 revealed two type III secretion systems (T3SS) on chromosomes 1 (T3SS1) and 2 (T3SS2) (26). T3SS is an apparatus used by several Gram-negative pathogenic bacteria to secrete and translocate virulence factor proteins into the cytosol of eukaryotic cells (27). The *V. parahaemolyticus* T3SS1 is analogous to the Ysc secretion system in *Yersinia*, and the *V. parahaemolyticus* T3SS2 is analogous to the Inv-Mxi-Spa secretion system in *Salmonella* and *Shigella* (28). The Ysc secretion system is typically related to cytotoxicity and the Inv-Mxi-Spa secretion system is associated with host cell invasion (28). In *V. parahaemolyticus* infection, the cellular dysfunction caused by T3SS-containing pathogens is remarkable. T3SS1 has been found in all isolated strains and is related to the cytotoxicity observed in HeLa cells. T3SS2 is found only in Kanagawa phenomenon (KP)-positive strains and produces enterotoxicity

that can be assayed using the rabbit ileal loop model (29). Recently, Kodama et al. identified two T3SS2 translocon proteins (VopB2, VopD2); translocon deletion strains lack the ability to cause fluid accumulation (30). Those studies strongly imply T3SS2 involvement in the enterotoxicity of *V. parahaemolyticus* and, consequently, the production of Vp-induced diarrhea.

In KP-positive strains, conserved T3SS2 genes are present on a pathogenicity island (Vp-PAI) in an 80-kb DNA region on chromosome 2 that also contains the TDH gene region (26, 31). Several T3SS2-secreted proteins have been implicated as potential virulence factors. VopA/P (VPA1346) has acetyltransferase activity that inhibits the binding of ATP to the mitogen-activated protein kinase (MAPK) kinase, resulting in an inactive kinase (32, 33). VopT (VPA1327) has ADP-ribosyltransferase (ADPRT) activity and ribosylates Ras, a member of the low-molecular-weight G proteins. In addition, VopT is partially responsible for the T3SS2-dependent cytotoxicity observed in Caco-2 cells (34). VopL (VPA1370), which has three Wiskott-Aldrich homology 2 domains, potently and directly facilitates the assembly of actin without any other eukaryotic factors (35). VopC (VPA1321) exhibits 38% homology to *Escherichia coli* cytotoxic necrotizing factor (34). But the role of these effectors in T3SS2-dependent fluid accumulation is unclear.

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