Virulence of Shiga Toxin-Producing *Escherichia coli* (STEC) in Orally-Infected Mice Correlates with the Type of Toxin Produced by the Infecting Strain

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OVERVIEW

In this chapter, we will provide a brief review of the attributes of the Shiga toxin (Stx) family with emphasis on the importance of these toxins in the pathogenesis of disease caused by *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC). We will also describe a modification of our streptomycin (str)-treated, orally-infected mouse model (23, 24) that permits the assessment of the relative virulence of various *E. coli* O157:H7 strains. In addition, we will illustrate how the type(s) of Stx produced by an *E. coli* strain is linked to virulence of that isolate when fed to str-treated mice. Lastly, we will underscore the role that activation of Stx2d by intestinal mucus plays in enhancing the pathogenicity of an infecting STEC strain that produces Stx2d.

BACKGROUND

Characteristics of Stxs

Shiga toxin produced by *Shigella dysenteriae* type 1, which is considered the prototype for the Stx family, was first described in 1903 by Conradi (2) and, independently, by Neisser and Shiga (14). Over the ensuing 95 years, Shiga toxin has been purified to homogeneity, characterized biologically and biochemically, and the genes encoding the toxin cloned and sequenced [reviewed in (17)]. In aggregate, these studies taken with X-ray crystallographic analysis (3) have culminated in a picture of Shiga toxin as a bi-partite molecule that is comprised of a single A polypeptide

of ~32 kDa and a pentamer of B polypeptides of ~7.7 kDa each. The A polypeptide can be nicked and reduced into an enzymatically active A₁ fragment and an A₂ fragment. The A₂ peptide functions to non-covalently link the A₁ polypeptide to the pentamer of B or receptor-binding polypeptides. The enzymatic activity of this potent cytotoxin for Vero and HeLa cells is that of an N-glycosidase that selectively removes a purine residue from 28S rRNA which, in turn, shuts down protein synthesis in the eucaryotic target cell (17). The receptor for Shiga toxin is globotriaosylceramide [(Gb₃) (17)].

Shiga toxin of *S. dysenteriae* type 1 (Stx) is the same toxin as Stx1 produced by many *E. coli* O157:H7 strains and related STEC (17). Most strains of *E. coli* O157:H7 also make Stx2. Stx1 and Stx2 share about 56 % deduced amino acid sequence homology, but polyclonal antiserum to Stx2 does not cross neutralize Stx1 or vice versa (17). Stx/Stx1, Stx2, and Stx2-related toxins have the same general structure and mode of action. However, Stx/Stx1 expression is repressed by high concentrations of iron, whereas Stx2 production is not iron-regulated. Some of the Stx family members also differ in specific toxic activity for tissue culture cells or for mice injected either intraperitoneally or intravenously with purified toxin preparations. Additionally, the virulence of STEC strains in orally-infected mice depends largely on the type of Stx produced by the infecting strain (discussed below).

Shiga toxin-producing *Escherichia coli* (STEC)

That certain diarrheagenic *E. coli* strains make a toxin related to Shiga toxin was first described by our laboratory in an abstract in 1978 (18) and subsequently confirmed in a peer-reviewed publication in 1982 (15). On a parallel tract, Konowalchuk and colleagues reported in 1978 that some *E. coli* isolates make a toxin that kills Vero cells (8). We subsequently showed that these Vero cell toxins and the Shiga toxins of *E. coli* were different names for the same proteins (16). Indeed, production of Stxs was ultimately demonstrated to be a hallmark trait of enterohemorrhagic *E. coli* (EHEC) O157:H7, the most common infectious cause of bloody diarrhea in the United States and the most frequent preceding event for the development of the hemolytic uremic syndrome (HUS) [see ref. (4) for a comprehensive review of *E. coli* O157:H7]. EHEC [so named by Levine (9)] are a subset of STEC that in addition to maintenance of a large plasmid and synthesis of Stxs also express the adhesin intimin. Intimin is encoded in the locus of enterocyte effacement (LEE), a newly described pathogenicity island that is also found in enteropathogenic *E. coli* (12). The epidemiological link between Stx production by an *E. coli*

isolate and the development of HUS after infection by that strain was established by Karmali and colleagues in their seminal papers (6, 7).

RELATIONSHIP BETWEEN RELATIVE TOXICITIES OF DIFFERENT STXS FOR MICE AND VIRULENCE OF STEC IN A MOUSE ORAL INFECTION MODEL

Mouse model for O157:H7 and non-O157 STEC

We previously showed that streptomycin (str)-treated outbred CD-1 mice succumbed to infection when fed 10^{10} organisms of a mouse-passaged strain of *E. coli* O157:H7 strain 933cu (cu for cured of its large plasmid), even though neither the parent 933cu strain nor 933 was virulent at 10^{10} organisms (23). We later found that this mouse-virulent strain, designated 933cu-rev, had acquired the capacity to grow on the distal small bowel epithelium and in mouse small intestinal mucus (23). We speculated that these acquired traits permitted increased systemic toxin delivery from the intestine compared to the parent 933cu strain. We also discovered that death of the mice fed 933cu-rev was due to tubular necrosis mediated by Stx2, but not Stx1, produced by the strain (24). Subsequently, we tested a panel of Stx1- and/or Stx2-producing *E. coli* O157:H7 strains as well as non-O157 strains in the str-treated CD-1 mouse model. We now conclude that an STEC strain is likely to be pathogenic in this CD-1 model if it either: i.) grows in small-intestinal mucus, colonizes the large intestine of the mice, and makes Stx2 or Stx2c [high oral 50% lethal dose (LD_{50})] or Stx2d (low oral LD_{50} , see below); or ii.) synthesizes very high levels of Stx2, Stx2c, or Stx2d (as expressed from a clone) and colonizes only the large bowel [see Table 1 as well as refs. (10, 24) for details on relative virulence of STEC strains for CD-1 mice]. More recently, we discovered that certain *E. coli* O157:H7 strains that make Stx1 and Stx2 or Stx2

Table 1. Correlation between toxicity of Stx type and virulence in mice

Toxin type	LD_{50} after intra-peritoneal injection of mice	Oral LD_{50} of STEC strain producing indicated toxin in str-treated		Activated by intestinal mucus
		CD-1 mice	DBA/2J mice	
Stx1	400 ng	Not virulent	Not virulent	No
Stx2	0.5-2 ng	$\sim 10^{10}$	$< 10^2$	No
Stx2c	ND	$\sim 10^{10}$	ND	No
Stx2d	1-5 ng	< 10	< 10	Yes
Stx2e	200 ng	Not virulent	ND ^{a)}	No

^{a)} ND, not determined.

Table 2. Virulence of O157:H7 strains in str-treated, orally-infected DBA/2J mice

Toxin (s) produced	No. virulent strains/ no. of strains tested
Stx1	0/2
Stx2	3/3
Stx1+2	1/3

Table 3. Stx2-specific antibody protects DBA/2J mice from lethal infection with an O157:H7 strain that produces both Stx1 and Stx2

Treatment ^{a)}	No. survivors/no. infected
PBS	0/5
MAb 13C4 (α -Stx1)	0/5
MAb 11E10 (α -Stx2)	5/5

^{a)} Administered to DBA/2J mice intraperitoneally (0.1ml) 24 hours before and 24 hr after oral infection with 10^9 CFU of O157:H7 strain 2812 (produces Stx1 and Stx2).

alone that are avirulent in str-treated CD-1 adult mice are pathogenic if fed to streptomycin-treated DBA/2J mice, Table 2. As with 933cu-rev in CD-1 mice, it is Stx2 that kills DBA/2J mice fed a virulent *E. coli* O157:H7 strain that produces both Stx1 and Stx2 (see Table 3). In fact, *E. coli* O157:H7 strains that make Stx1 only are avirulent in DBA/2J mice. That Stx2 is about 400-fold more toxic for mice when injected parenterally than is Stx1 [(21) and summarized in Table 1] probably explains the differences in virulence among these strains. Of note is the correlation between this observation that Stx2 and Stx2-related toxins contribute to virulence of STEC in a mouse model whereas Stx1 does not, and the finding that Stx2-producing *E. coli* O157:H7 more frequently cause HUS than those that make Stx1 only (19). The fact that certain O157:H7 strains that produce Stx2 or Stx2 and Stx1 are virulent in DBA/2J mice but not in CD-1 mice could be explained by differences in the size of the animals. Indeed, we speculate that virulence in this model is not only due to type of toxin but also level of toxin produced relative to body weight of the mouse. Four observations support various aspects of this hypothesis: i.) male DBA/2J mice weigh about 17 g while adult CD-1 mice weigh about 24 g; ii.) a str-resistant *E. coli* K-12 strain transformed with a multicopy plasmid encoding Stx2 or Stx2d is sufficient to cause kidney damage and kill orally-infected adult CD-1 mice (11, 24); iii.) EHEC O157:H7 strain 86-24 (Stx2-producer) has a low LD₅₀ in adult

DBA/2J animals but a high LD₅₀ in adult CD-1 mice (see Table 1); and, iv.) adult inbred mice of approximately the same size as DBA/2J mice (C57BL/6J, BALB/cJ, and C3H/HeJ) are also susceptible to lethal infection with strain 86-24 (unpublished observation). Whatever the actual basis of the sensitivity of adult DBA/2J mice to many Stx2- or Stx1- and Stx2-producing *E. coli* O157:H7 strains, we propose that this mouse model is appropriate for evaluating relative virulence of such isolates.

Enhanced virulence of Stx2d-producing non-O157 strains in the CD-1 mouse model

When we began to screen various strains of STEC for mouse virulence, we made the startling discovery that STEC O91:H21 strain B2F1 killed str-treated adult CD-1 mice when fed at doses as low as 10 organisms (10). We also identified another O91:H21 strain, designated H414-36/89 (10), that was similarly virulent for these large mice. We proposed that the extraordinary pathogenicity of these isolates might be due to the production Stx2d toxins [originally called Stx2vh, see ref. (10)] by these strains. In fact, we later showed that the toxicity of Stx2d can be increased (activated) by exposure to mouse or human colonic mucus and that this capacity for activation is unique to the Stx2d variant of Stx2 [see Table 1 and ref. (13)]. More recently, we identified other non-O157 STEC strains that are highly virulent for str-treated CD-1 mice [e.g. an O104:H21 isolate, 3024-94, that caused an outbreak of hemorrhagic colitis in the U.S. in 1994 (1)] and found that they also make Stx2d (unpublished finding). To date Stx2d is the only Stx that we have identified which can be activated by intestinal mucus. The only amino acid differences that can account for the fact that Stx2d is activatable but not Stx2 or Stx2c (13) are two amino acids in the C-terminus of the A₂ fragment of those toxins (13). We are in the process of identifying which of these amino acids are critical for activation and defining the biochemical basis for activation. We also intend to explore how activation actually translates into increased toxicity.

CONCLUSION

The type of Stx produced by an infecting STEC strain directly relates to its virulence in a str-treated orally infected mouse model: Stx/Stx1 is less toxic than Stx2 or Stx2c, and Stx2 and Stx2c are less toxic than Stx2d. Adult CD-1 mice are less sensitive than DBA/2J mice to these toxins as delivered following oral infection; hence, the DBA/2J model can be used to screen Stx2⁺ O157:H7 strains for relative virulence. Finally,

the low oral LD₅₀ of non-O157 isolates that make Stx2d appears to be directly related to the fact that the toxicity of these proteins increases in the presence of intestinal mucus.

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