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Relationship of Genetic Type of Shiga Toxin to Manifestation of Bloody Diarrhea due to Enterohemorrhagic Escherichia coli Serogroup O157 Isolates in Osaka City, Japan

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One hundred sixty-nine strains of enterohemorrhagic Escherichia coli serogroup O157 were examined for the correlation between the genotype of their Shiga toxin genes (stx) and manifestation of bloody diarrhea (BD). It was shown that the strains carrying only stx2vha were probably less virulent and caused BD less frequently.

Enterohemorrhagic Escherichia coli (EHEC) of serogroup O157 was recognized as the causative agent following two outbreaks of hemorrhagic colitis (HC) in 1982 (12). The organisms are characterized by their ability to elaborate Shiga toxin (Stx) (2, 8). The association of EHEC with HC and hemolytic-uremic syndrome (HUS) implies that Stx is a major virulence factor in these diseases (4, 11, 15, 17). However, the role of Stx in the pathogenesis of HC remains to be elucidated. Tzipori et al. showed that Stx-negative variant strains still caused diarrhea (19). Attempts to link Stx with clinical manifestations of HC have been performed with epidemiological, clinical, and animal model investigations (6, 7, 10). However, these studies have been performed prior to the discovery of Stx2 by Scotland et al. (14). Hence, it is not clear from reading the original sources whether single or multiple forms of Stx were being produced.

It was shown that the strains carrying only stx2vha were positive for stx2vhb, stx2e, or stx2ev in this study. This is concordant with the study of Tyler et al., in which stx2vhb was found only among non-O157 Stx-producing E. coli strains (18). Symptoms of BD were reported for 50% (48 of 96) of patients from whom isolates possessing both stx1 and stx2 were isolated; BD was defined as diarrhea that contained blood that could be observed macroscopically. BD was reported for 55% (6 of 11), 39% (9 of 23), 18% (2 of 11), and 8% (2 of 25) of patients infected by isolates possessing only stx2vha, both stx1 and stx2vha, both stx1 and stx2va, and only stx2va, respectively (Table 1). After the strains were assigned to three groups on the basis of Stx2 genotypes, these results indicated that patients with strains that possessed stx2 or both stx2 and stx2va often presented with BD. On the other hand, the patients from whom

<table>
<thead>
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<th>RPLA titer with anti-stx2 latex</th>
<th>No. of BD patients with indicated genotype/total infected</th>
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</tr>
<tr>
<td>2,560 4 6 2,560 1</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

Subtotal 48/96 6/11 9/23 2/11 2/25 1/2

* One strain possessed only stx1.

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organisms having only \textit{stx2vha} were isolated manifested BD with lower frequency (Table 2).

Significant differences were not observed in the ages and sexes of patient groups from which each \textit{stx} type of organism was isolated (Table 2); however, the patients with BD were significantly younger than infected persons without BD (Table 3). It was not suggested that particular phage and/or DNA types were related to manifestation of BD (data not shown).

Why the strains that possessed only \textit{stx2vha} caused BD less frequently remains to be elucidated. However, it may reflect functional difference between Stx2 and Stx2vha. Stx1 and Stx2 bind preferentially to Gb3 [Gal\textsubscript{a}(1\rightarrow4)Gal\textsubscript{b}(1\rightarrow4)Glc-ceramide] (20), while Stx2vha and Stx2e bind preferentially to Gb4 [GalNAc\textsubscript{b}(1\rightarrow3)Gal\textsubscript{a}(1\rightarrow4)Gal\textsubscript{b}-(1\rightarrow4)Glc-ceramide] (13). Takeda et al. observed that Vero cell cytotoxicity and mouse lethality of Stx2vh were somewhat lower than those of Stx2 (16). Tyler et al. (18) also reported that the culture supernatants of strains carrying only Stx2 variant genes gave low cytotoxin titers in HeLa cell assays. Differences in these biological activities can possibly explain why the strains that possessed only \textit{stx2vha} caused BD less frequently.

Alternatively, it was possible that the quantity of Stx produced was an important factor. The amount of Stx that was reactive to the anti-Stx2 latex particles depended on the Stx genotype. Strains possessing \textit{stx2vha} tended to have lower titers than strains with \textit{stx2}, regardless of the presence or absence of \textit{stx1} (Fig. 1). It is possible that the anti-Stx2 latex was less reactive to Stx2vha. To address this, gene sequences in strains possessing only \textit{stx2vha} were preliminarily examined. No difference was found in the sequence of \textit{stx2vha} between two strains that produced high titers and two with low titers (data not shown), suggesting that Stx2vha molecules produced by these strains were identical. Therefore, the low titers expressed by the majority of strains possessing only \textit{stx2vha} seemed to reflect a lower production of Stx2vha. If so, the discrepancy in manifestation of BD could be due to the Stx levels produced by each strain.

It appeared that the strains carrying \textit{stx2} tended to cause BD more than strains possessing only \textit{stx2vha}. Classical HUS develops typically a few days after the onset of an acute diarrheal prodromal illness, which is often bloody. In cases of patients infected with EHEC O157, it may be that HUS is also caused by organisms having \textit{stx2} or both \textit{stx2} and \textit{stx2vha} rather than bacteria with only \textit{stx2vha}, although BD is not an essential symptom for HUS. In fact, all six strains isolated from HUS

![FIG. 1. Relationship between Stx2 RPLA titer and the genotype of EHEC O157 strains. One hundred sixty-eight strains of EHEC O157 were assigned to three groups on the basis of their types of Stx2 genes. Proportions of strains that showed each RPLA titer in the genogroup are indicated.](image-url)
patients who first contracted BD in this study possessed both stx1 and stx2. Ostroff et al. suggested that EHEC O157 strains that produced Stx2 alone were more likely to be associated with the development of HUS than strains that produced Stx1 alone or Stx1 and Stx2 (9). In the present investigation, however, the possession of stx1 did not have any negative influence on manifestation of BD. BD was found more among patients infected by strains with both stx1 and stx2vha (18%) than among those infected by strains with stx2vha alone (8%) (Table 1). Of 18 strains that did not possess stx1 and gave a low RPLA titer of Stx2 (<80), only one was from a patient with BD. On the other hand, 6 of 22 strains that gave an Stx2 titer of less than 80 but had stx1 were from patients with BD. These differences, however, were not statistically significant, and the role of Stx1 in BD could not be evaluated since there was only a single strain that produced only Stx1 in this study.

In conclusion, the present study indicates that the typing of Stx2 genes of EHEC O157 isolates provides useful information not only for epidemiological analysis but for prognosis, as strains carrying only stx2vha are possibly less virulent and cause BD less frequently.

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REFERENCES