© 2009 International Society of Nephrology

Host and pathogen determinants of verocytotoxin-producing *Escherichia coli*-associated hemolytic uremic syndrome

Mohamed A. Karmali[1]

[1]*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario, Canada*

Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) infection is associated with a spectrum of clinical manifestations that includes diarrhea, hemorrhagic colitis, and the hemolytic uremic syndrome (HUS). The occurrence of HUS in a minority of individuals in outbreaks of VTEC infection is a function of several pathogen and host factors. Pathogen factors include the inoculum size and serotype of the infecting strain, horizontally acquired genetic elements known as pathogenicity islands, and probably the VT type. Host factors that increase the risk of developing HUS include age, pre-existing immunity, gastric acidity, the use of antibiotics and anti-motility agents, and, probably, stress and genetic factors that modulate host response to infection, such as innate immunity and toxin receptor type, expression, and distribution. A better understanding of the pathogen and host determinants of HUS can aid in the development of more effective public health strategies to reduce the risk of developing HUS.

Kidney International (2009) **75** (Suppl 112), S4–S7; doi:10.1038/ki.2008.608

KEYWORDS: *Escherichia coli*; hemolytic uremic syndrome; verocytotoxin; virulence; hemorrhagic colitis

Correspondence: *Mohamed A. Karmali, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, 110 Stone Road West, Guelph, Ontario, Canada N1G 3W4. E-mail: mohamed_karmali@phac-aspc.gc.ca*

Verocytotoxin (VT)-producing *Escherichia coli* (VTEC) infection is associated with a spectrum of clinical manifestations that includes diarrhea, hemorrhagic colitis, and the hemolytic uremic syndrome (HUS).[1–3] Systemic VT toxemia is considered to be central to the genesis of HUS.[3] Although over 200 different OH serotypes of VTEC have been isolated from cases of human illness,[4] only O157:H7 and a handful of other serotypes are associated with HUS,[5] suggesting that bacterial factors other than VTs also contribute to the development of HUS. The whole genome sequences of two *E. coli* O157:H7 outbreak strains[6,7] have revealed a large number of candidate virulence factors, often present on pathogenicity islands (PAIs), that may contribute to the development of this syndrome. During outbreaks of *E. coli* O157:H7 infection, only a proportion of infected individuals develop HUS,[8] suggesting that, in addition to pathogen factors, host factors also contribute to its development. *E. coli* O157:H7 is evolving and diversifying rapidly.[9] Some O157:H7 strains appear to be non-pathogenic for humans,[10] whereas others may have developed an enhanced propensity to cause HUS.[11] The purpose of this paper is to review the disease mechanisms of VTEC with special reference to the pathogen and host determinants of HUS.

PATHOGEN DETERMINANTS OF HUS

The low infectious dose of *E. coli* O157:H7 (~100–500 organisms)[12] is a major determinant of its ability to cause severe and epidemic disease, although the underlying mechanisms for this are not fully understood. Gastric acid is an important first barrier to ingested pathogens, and thus the reported resistance of the organism to gastric acid[13] helps to explain the low infectious dose.

Over 200 different serotypes of VTEC have been associated with human disease, but outbreaks of disease and HUS have been associated only with serotype O157:H7 and occasionally with a handful of non-O157 serotypes, such as O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM. The explanation for why only a restricted number of serotypes are associated with HUS has been unclear. However, growing evidence suggests that a major pathogen determinant of serotypes that are associated with outbreaks and HUS is the presence of specific horizontally acquired gene cassettes

known as PAIs. The best known PAI is the locus of enterocyte effacement (LEE),[14–16] which allows the organism to colonize the mucosal epithelial cells of, probably, the large bowel with a characteristic 'attaching and effacing' cytopathology.[17,18] LEE encodes the structural, accessory, and effector molecules of a type III secretion system (TTSS),[19] which is a macromolecular complex spanning both bacterial membranes that is used by many Gram-negative bacterial pathogens to inject virulence factors directly into host cells to subvert host cellular function for the benefit of the pathogen.[20] However, LEE cannot alone be the determinant of outbreaks and HUS because several VTEC serotypes that are not associated with HUS and outbreaks are also LEE-positive. On the other hand, the type III secretion system also secretes many other effector molecules, encoded outside the LEE on other PAIs, that are referred to as non-LEE-encoded effectors (Nle's).[21,22] At least three Nle-encoding PAIs (OIs 57, 71, and 122) have now been linked to non-O157 VTEC that cause HUS,[23] and the critical virulence function of the proteins they encode, especially in OI-122, is being elucidated in experimental animal models.[24] It is thus becoming clear that a number of PAIs, including LEE, play a major role in enhancing the ability of various serotypes to cause HUS. It should be noted that some O157:H7 strains appear to be non-pathogenic for humans,[10] whereas others may have developed an enhanced propensity to cause HUS.[11] However, the genetic basis for these observations has not been fully elucidated.

Human VTEC strains elaborate at least four potent bacteriophage-mediated VTs: VT1, VT2, VT2c, and VT2d.[16,25] Each may be present alone, or in a combination of two or three different VTs. VT1 is virtually identical to Shiga toxin, but is serologically distinct from VT2c.[3,26] The toxins share a common polypeptide subunit structure, consisting of an enzymatically active A subunit ($\sim$32 kDa) linked to a pentamer of B subunits ($\sim$7.5 kDa). VTs are produced in the bowel and are translocated intact into the circulation, although the mechanisms of toxin translocation and distribution to target endothelial cells in the renal glomeruli, gastrointestinal tract, pancreas, and other organs and tissues[27] are not fully understood.[28,29] After binding to the glycolipid receptor globotriaosylceramide (Gb3)[30] on the endothelial cell, the toxins are internalized by receptor-mediated endocytosis.[31] They then target the endoplasmic reticulum through the Golgi by a process termed 'retrograde transport.'[31] Inside the host cell, the A subunit is proteolytically nicked to give an enzymatically active A_1 fragment,[26] which cleaves the N-glycosidic bond at position A4324 of the 28S rRNA of the 60S ribosomal subunit.[32] This blocks elongation factor-1-dependent aminoacyl tRNA binding, resulting in the inhibition of protein synthesis.[26] VTs may also damage eukaryotic cells by apoptosis.[33] Cytokines, especially TNF (tumor necrosis factor)-α and IL1 (interleukin 1)-β, potentiate toxin action through upregulation of the cellular receptor, Gb3. It is thought that increased cytokine production might be the result of VT action on monocytes.[33]

The different VTs show differences in specific binding affinities and cytotoxic activities in cell culture,[26,34] as well as in tissue specificities[35,36] and clinical syndromes in experimental animals.[37,38] Two or more binding sites for Gb3 have been recognized on the VT1 B subunit,[39] and the VT/Gb3 interaction is influenced by the length of the fatty acid side chain *in vitro*.[40] The clinical and pathophysiological implications of different toxin type and Gb3 conformations are not fully understood. However, there is evidence that VT2 is associated with more severe disease in humans,[41] and, further, that it may enhance gut colonization by VTEC.[42] VT2c has been speculated to contribute to enhanced disease severity associated with an emerging clone of *E. coli* O157:H7.[11]

HOST DETERMINANTS OF HUS

Several host factors influence the risk of acquiring VTEC infection and of developing HUS, including behavioral factors (such as eating undercooked hamburger), age,[43] immunity,[44] health status,[45] the use of antibiotics and anti-motility agents,[46] stress, and genetic factors.

The highest age-specific frequency of VTEC-associated HUS is in infants and young children.[43] The age-specific frequency declines with increasing age and increases again in the elderly. This age distribution of HUS correlates inversely with the age-specific frequency of antibodies to VT1 and VT2,[44] suggesting that pre-existing immunity plays a significant role in host resistance to HUS.

Gastric acidity is an important initial host barrier to ingested pathogens. Evidence for its protective role against *E. coli* O157:H7 infection is that individuals with low gastric acidity (for example, owing to gastrectomy or pernicious anemia) are at a significantly higher risk of developing HUS than those with normal physiological gastric function.[45]

The genes that regulate colonization of the bowel by *E. coli* O157:H7 may be modulated by hormone-like soluble factors produced by other bacterial cells in a density-dependent manner in a process referred to as 'quorum sensing.'[47] Interestingly, the quorum sensing pathway can also be activated by host stress hormones such as epinephrine and norepinephrine.[48,49] The pathophysiological implications of this, as well as the possible role of stress as a risk factor for severe disease, are under study.

Host genetic factors may influence host–pathogen interactions, including the innate immune response to infection and the nature of the toxin–cell interaction. However, knowledge of this is in its infancy.

CONCLUDING REMARKS

A better understanding of the pathogen and host determinants of HUS can aid in the development of more effective public health strategies to reduce the risk of developing HUS. For example, knowledge of specific pathogen risk factors for HUS can contribute to the identification of potential vaccine candidates as well as to the improvement of diagnosis and surveillance of high-risk VTEC to allow for more rapid

recognition and containment of outbreaks, thus reducing morbidity and mortality. Similarly, knowledge of host risk factors, such as the use of antibiotics and anti-motility agents, can help to modify behaviors that can mitigate the risk of HUS.

DISCLOSURE

The author has declared no financial interests.

REFERENCES

1. Karmali MA, Petric M, Steele BT *et al.* Sporadic cases of hemolytic-uremic syndrome associated with fecal cytotoxin and cytotoxin-producing *Escherichia coli*. *Lancet* 1983; **1**: 619–620.
2. Karmali MA, Petric M, Lim C *et al.* The association between hemolytic uremic syndrome and infection by Verotoxin-producing *Escherichia coli*. *J Infect Dis* 1985; **151**: 775–782.
3. Karmali MA. Infection by Verocytotoxin-producing *Escherichia coli*. *Clin Microbiol Rev* 1989; **2**: 15–38.
4. World Health Organization. *Zoonotic non-O157 Shiga toxin-producing Escherichia coli (STEC)*. Report of a WHO Scientific Working Group Meeting; 22–23 June 1998; Berlin, Germany. WHO/CSR/APH/98.8, 1999; pp 1–30.
5. Karmali MA, Mascarenhas M, Shen S *et al.* Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol* 2003; **41**: 4930–4940.
6. Hayashi T, Makino K, Ohnishi M *et al.* Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res* 2001; **8**: 11–22.
7. Perna NT, Plunkett III G, Burland V *et al.* Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature* 2001; **409**: 529–533.
8. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* 1991; **13**: 60–98.
9. Wick LM, Qi W, Lacher DW *et al.* Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. *J Bacteriol* 2005; **187**: 1783–1791.
10. Kim J, Nietfeldt J, Benson AK. Octamer-based genome scanning distinguishes a unique subpopulation of *Escherichia coli* O157:H7 strains in cattle. *Proc Natl Acad Sci USA* 1999; **96**: 13288–13293.
11. Manning SD, Motiwala AS, Springman AC *et al.* Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. *Proc Natl Acad Sci USA* 2008; **105**: 4868–4873.
12. Griffin PM. Epidemiology of shiga toxin-producing *Escherichia coli* infections in humans in the United States. In: Kaper JB, O'Brien AD (eds). *Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains*. ASM Press: Washington, DC, 1998, pp 15–22.
13. Lin J, Smith MP, Chapin KC *et al.* Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl Environ Microbiol* 1996; **62**: 3094–3100.
14. Jerse AE, Yu J, Tall BD *et al.* A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci USA* 1990; **87**: 7839–7843.
15. McDaniel TK, Jarvis KG, Donnenberg MS *et al.* A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci USA* 1995; **92**: 1664–1668.
16. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; **11**: 142–201.
17. Knutton S, Lloyd DR, McNeish AS. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. *Infect Immun* 1987; **55**: 69–77.
18. Moon HW, Whipp SC, Arzenio RA *et al.* Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect Immun* 1983; **41**: 1340–1351.
19. Foubister V, Rosenshine I, Donnenberg MS *et al.* The eaeB gene of enteropathogenic *Escherichia coli* is necessary for signal transduction in epithelial cells. *Infect Immun* 1994; **62**: 3038–3040.
20. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol Mol Biol Rev* 1998; **62**: 379–433.
21. Deng W, Puente JL, Gruenheid S *et al.* Dissecting virulence: systematic and functional analyses of a pathogenicity island. *Proc Natl Acad Sci USA* 2004; **101**: 3597–3602.
22. Garmendia J, Frankel G, Crepin VF. Enteropathogenic and enterohemorrhagic *Escherichia coli* infections: translocation, translocation, translocation. *Infect Immun* 2005; **73**: 2573–2585.
23. Coombes BK, Wickham ME, Mascarenhas M *et al.* Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing Escherichia coli strains. *Appl Environ Microbiol* 2008; **74**: 2153–2160.
24. Wickham ME, Lupp C, Mascarenhas M *et al.* Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *J Infect Dis* 2006; **194**: 819–827.
25. Melton-Celsa AR, O'Brien AD. Structure, biology, and relative toxicity of Shiga toxin family members for cells and animals. In: Kaper JB, O'Brien AD (eds). *Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains*. ASM Press: Washington, DC, 1998, pp 121–128.
26. O'Brien AD, Tesh VL, Donohue-Rolfe A *et al.* Shiga toxin: biochemistry, genetics, mode of action and role in pathogenesis. *Curr Top Microbiol Immunol* 1992; **180**: 65–94.
27. Richardson SE, Rotman TA, Jay V *et al.* Experimental verocytotoxemia in rabbits. *Infect Immun* 1992; **60**: 4154–4167.
28. Hurley BP, Jacewicz M, Thorpe CM *et al.* Shiga toxins 1 and 2 translocate differently across polarized intestinal epithelial cells. *Infect Immun* 1999; **67**: 6670–6677.
29. Philpott DJ, Ackerley CA, Kiliaan AJ *et al.* Translocation of verotoxin-1 across T84 monolayers: mechanism of bacterial toxin penetration of epithelium. *Am J Physiol* 1997; **273**(Part 1): G1349–G1358.
30. Lingwood CA, Law H, Richardson SE *et al.* Glycolipid binding of natural and recombinant *Escherichia coli* produced Verotoxin *in-vitro*. *J Biol Chem* 1987; **262**: 8834–8839.
31. Sandvig K, van Deurs B. Endocytosis, intracellular transport, and cytotoxic action of Shiga toxin and ricin. *Physiol Rev* 1996; **76**: 949–966.
32. Endo Y, Tsurugi K, Yutsudo T *et al.* Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of shiga toxin on eukaryotic ribosomes; RNA N-glycosidase activity of the toxins. *Eur J Biochem* 1988; **171**: 45–50.
33. Monnens L, Savage CO, Taylor CM. Pathophysiology of hemolytic-uremic syndrome. In: Kaper JB, O'Brien AD (eds). *Escherichia coli O157:H7 and Other Shiga Toxin-Producing E. coli Strains*. ASM Press: Washington, DC, 1998 pp 287–292.
34. Head SC, Karmali MA, Lingwood CA. Preparation of VT1 and VT2 hybrid toxins from their purified dissociated subunits. *J Biol Chem* 1991; **266**: 3617–3621.
35. Bielaszewska M, Clarke I, Karmali MA *et al.* Localization of intravenously administered verocytotoxins (shiga-like toxins) 1 and 2 in rabbits immunized with homologous and heterologous toxoids and toxin subunits. *Infect Immun* 1997; **65**: 2509–2516.
36. Ludwig K, Karmali MA, Smith CR *et al.* Cross-protection against challenge by intravenous *Escherichia coli* verocytotoxin 1 (VT1) in rabbits immunized with VT2 toxoid. *Can J Microbiol* 2002; **48**: 99–103.
37. Baker DR, Moxley RA, Steele MB *et al.* Differences in virulence among *Escherichia coli* O157:H7 strains isolated from humans during disease outbreaks and from healthy cattle. *Appl Environ Microbiol* 2007; **73**: 7338–7346.
38. Head SC, Petric M, Richardson S *et al.* Purification and characterization of verocytotoxin 2. *FEMS Microbiol Letts* 1988; **51**: 211–216.
39. Bast DJ, Banerjee L, Clark C *et al.* The identification of three biologically relevant globotriaosyl ceramide receptor binding sites on the Verotoxin 1 B subunit. *Mol Microbiol* 1999; **32**: 953–960.
40. Kiarash A, Boyd B, Lingwood CA. Glycosphingolipid receptor function is modified by fatty acid content. Verotoxin 1 and verotoxin 2c preferentially recognize different globotriaosyl ceramide fatty acid homologues. *J Biol Chem* 1994; **269**: 11138–11146.
41. Boerlin P, McEwen SA, Boerlin-Petzold F *et al.* Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol* 1999; **37**: 497–503.
42. Robinson CM, Sinclair JF, Smith MJ *et al.* Shiga toxin of enterohemorrhagic *Escherichia coli* type O157:H7 promotes intestinal colonization. *Proc Natl Acad Sci USA* 2006; **103**: 9667–9672.
43. Rowe PC, Orrbine E, Wells GA *et al.* Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. The Canadian Pediatric Kidney Disease Reference Centre. *J Pediatr* 1991; **119**: 218–224.
44. Karmali MA, Mascarenhas M, Petric M *et al.* Age-specific frequencies of antibodies to *Escherichia coli* Verocytotoxins (Shiga Toxins) 1 and 2 among urban and rural populations in Southern Ontario. *J Infect Dis* 2003; **188**: 1724–1729.
45. Carter AO, Borczyk AA, Carlson JAK *et al.* A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. *N Engl J Med* 1987; **317**: 1496–1500.

46. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005; **365**: 1073–1086.
47. Clarke MB, Hughes DT, Zhu C *et al*. The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci USA* 2006; **103**: 10420–10425.
48. Hughes DT, Sperandio V. Inter-kingdom signalling: communication between bacteria and their hosts. *Nat Rev Microbiol* 2008; **6**: 111–120.
49. Sperandio V, Torres AG, Jarvis B *et al*. Bacteria–host communication: the language of hormones. *Proc Natl Acad Sci USA* 2003; **100**: 8951–8956.