Epidemiology and spectrum of disease of *Escherichia coli* O157

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Purpose of review

The Shiga toxin-producing *Escherichia coli* strains, such as *E. coli* O157:H7, have emerged as major diarrheal pathogens both in the United States and elsewhere. These organisms are important because gastrointestinal infection (afebrile hemorrhagic colitis) can trigger microangiopathic hemolytic anemia and renal failure (hemolytic uremic syndrome). Understanding the pathophysiology of this illness is likely to lead to important new treatment interventions.

Recent findings

It is now recognized that children with hemorrhagic colitis routinely develop a spectrum of coagulation abnormalities and that only a fraction of children develop full blown hemolytic uremic syndrome. Individual variability in expression of inflammatory mediators is likely to be a key element in determining which children progress to the severe end of the spectrum of disease. The value of antibiotic therapy is unknown.

Summary

The pathophysiology of HUS remains incompletely understood. The lag between onset of diarrhea and onset of HUS represents an opportunity to intervene and prevent renal failure. However, there currently is no way to prevent such life threatening complications. The management should focus on diagnosis and close observation so that early intervention can prevent complications.

Keywords

hemolytic uremic syndrome, Shiga toxin producing *E. coli*, microangiopathic hemolytic anemia

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Abbreviations

HUS	hemolytic uremic syndrome
Stx	Shiga toxin
STEC	Shiga toxin producing *Escherichia coli*

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Introduction

Hemolytic uremic syndrome (HUS) is defined by the presence of microangiopathic hemolytic anemia, acute renal failure, and thrombocytopenia. The cause of the vast majority of cases is gastrointestinal infection by Shiga toxin (Stx) producing *Escherichia coli* (STEC). These bacteria are sometimes called verotoxin-producing *E. coli* (VTEC), Shiga-like toxin-producing *E. coli*, and enterohemorrhagic *E. coli* (EHEC).

In the United States and Canada, most HUS cases are associated with a single serotype (*E. coli* O157:H7) although multiple other STEC serotypes cause HUS. Non-O157 types vary geographically in importance. For example, in Austria and Germany they are associated with 43% of STEC positive HUS patients [1•]. In Australia O157:H7 is rare and O111 strains are the common STEC type [2]. In contrast, in the US more than 80% of STEC infections are by O157 strains [3].

Gastrointestinal phase of Shiga toxin-producing *Escherichia coli* illness

Infection can be symptomatic or asymptomatic. Family contacts of index cases often have evidence of asymptomatic infection [4•]. The initial clinical signs of infection in those who become ill include severe abdominal pain, vomiting, and watery diarrhea. Within the first several days of illness the diarrhea becomes blood streaked or grossly bloody. Despite this evidence of colitis, the children typically have little or no fever. This constellation of signs and symptoms is called hemorrhagic colitis. At the most severe end of the spectrum ischemic colitis and perforation can occur [5]. Colonic stenosis can develop after ischemic colitis. Although colonoscopy is infrequently done, when performed it shows either diffuse severe inflammation with edema and friability, or inflammatory changes primarily in the right colon, or longitudinal ulcers [6]. Cholelithiasis necessitating cholecystectomy, persistent pancreatitis, and glucose intolerance are additional potential gastrointestinal sequelae [7].

Risk of hemolytic uremic syndrome

Using strict criteria for diagnosis only 5–8% of children with toxin associated diarrhea develop HUS. However, there has been tremendous variability in reported risk. There were 173 cases of *E. coli* O157 in England and Wales from 1992 to 1994 of which 21% developed HUS [8]. In an outbreak of O157 in the Inuit communities in the Canadian Northwest Territories, 22 of 152 (14%) patients infected with O157 developed HUS [9]. In the

large Pacific Northwest outbreak the data suggested that of the 501 cases, 45 developed HUS (9%) [10]. In contrast, an outbreak in Japan caused illness in 12 680 children but only 1% progressed to HUS [11]. When the standard criteria have been modified to include cases that have minimal evidence for HUS, the risk is about 15–18% [12]; these additional cases probably represent a part of the spectrum previously referred to as incomplete or forme fruste HUS [13,14]. High white blood cell counts early in illness and elevated C reactive protein levels are useful in predicting an increased risk of HUS [14–18]. Although fever is typically not found in hemorrhagic colitis, when it occurs the patient is at increased risk of developing HUS [19]. Hypochlorhydria and age above 65 years are risk factors for HUS; the risk of death is highest in the elderly [14].

Epidemiology

For over 20 years it has been recognized that intestinal infection of cattle can cause STEC to contaminate the food consumed by humans [20]. Hamburgers in particular have been the cause of multiple outbreaks of disease [21•]. Infection persists in animal reservoirs in part because the organism survives in soil for long periods of time [22]. Other animals, including pigs [23], sheep [24], and deer [25], can also carry STEC and many other foods and water can be contaminated with the feces of infected animals. The result has been outbreaks involving municipal water supplies [26], venison [27], uncooked vegetables, cheese curd [28], apple cider, and sprouts [29]. In addition, because incredibly low numbers of these organisms can cause illness, person-to-person spread within families and in day care centers has resulted in further propagation of disease. The majority of family contacts of a child with HUS have evidence of infection as indicated by antibodies to toxins and O157 lipopolysaccharide [4•]. Over 80% of family contacts have evidence of infection as indicated by demonstration of Stx bound to their blood polymorphonuclear leukocytes [30]. Even brief visits to dairy farms and petting zoos have been incriminated as potential risk factors for children [31–33]. The low-level exposure associated with swimming in contaminated water can lead to serious infection [34].

Pathophysiology

There are two major toxins: Stx1 and Stx2. Stx1 made by *E. coli* is essentially identical to Shiga toxin, the protein synthesis-inhibiting toxin of *Shigella dysenteriae* serotype 1. Stx2 has multiple variants (Stx2, Stx2c, Stx2d, Stx2e, Stx2f) that are in general closely related to each other but more distantly related to Stx1 (55–60% amino acid homology between Stx1 and the various types of Stx2). Some STEC strains produce only Stx1, some Stx2 (or variants of Stx2), while still others produce both toxins. A few strains produce as many as three toxin variants [35]. The toxins do not appear to carry equivalent risks of causing HUS. Strains that produce only Stx1 have the lowest risk, while strains that produce only Stx2 have the highest risk; strains that make both Stx1 and Stx2 carry an intermediate risk. However, genotype variants Stx2d and Stx2e appear to have little or no risk of causing HUS [36••]. Stx1 variants are uncommon although an ovine variant, Stx1c, can be isolated from asymptomatic or mildly ill humans [37•]. Typically the strains that cause illness in humans also possess genes that aid in intestinal colonization via intimin-mediated attaching-effacing lesions [36••], although infrequently other adhesins may be important [38]. Production of Shiga toxins is not limited to *E. coli* and *S. dysenteriae*. Other intestinal Gram-negative rods sometimes produce these toxins although their link to human disease is currently less clear than that for STEC.

Shiga toxins are protein synthesis inhibiting toxins that bind to genetically determined surface-expressed neutral glycolipids containing a trisaccharide, Gal α [1→4] Gal β [1→4] Glc linked to ceramide. This glycolipid is variously referred to as Gb_3, CD77 or P^k. Renal tubule and glomerular Gb3 expression may be higher in infants than adults, perhaps explaining in part the age-related susceptibility [39]. The toxins have five identical copies of receptor-binding B subunits and one copy in the enzymatically active A subunit. The B subunit has the additional ability to induce apoptosis of at least some cell types. The A subunit cleaves an adenine residue of 28S ribosomal RNA at the site of elongation factor-1-dependent aminoacyl transfer RNA attachment to irreversibly inhibit protein synthesis. It is unclear whether both protein synthesis inhibition and apoptosis are relevant to human disease.

Injury to vascular endothelial cells is generally thought to be the central event in the pathogenesis of HUS [40,41]. Ultrastructural examination of capillaries in tissues from patients with HUS reveals a characteristic swelling of vascular endothelial cells accompanied by widening of the subendothelial space and intravascular fibrin/platelet thrombi. Endothelial models of HUS have sometimes yielded contradictory and confusing results because isolated vascular endothelial cells behave differently *in vitro* depending on their source [42,43].

The toxin injury model hypothesizes that Stx (and perhaps endotoxin) produced in the gut are taken up; these toxins induce proinflammatory cytokines that up-regulate Gb_3 receptors on vascular endothelial cells allowing Stx to bind and subsequently injure endothelial cells. Stx1 and Stx2 have been demonstrated in the kidney of a child who died from HUS [44] suggesting that direct toxin injury is likely to be the mechanism underlying development of HUS.

Although vascular endothelial injury is believed to be the central event in the pathophysiology of Stx-associated HUS, the role of host immunity, toxin receptor expression, and intravascular coagulation in the development vascular injury is uncertain. Conventional wisdom about pathogenesis has been based heavily on tissue culture models, animal models, and findings in children who have already developed HUS [45••].

Animal models including those using mice, rabbits, greyhounds (Alabama rot), chickens (swollen head syndrome), and pigs (edema disease), have been inadequate for study of the vascular endothelial events that result in HUS. However, the baboon model promises to give important new insights into pathogenesis because it appears to mimic the vascular and renal events of human HUS. For example, the role of prostacyclin has long been debated in HUS. Using the baboon model it was shown that prostacyclin is very unlikely to be central to pathophysiology [46••]. Likewise, studies using this model also strongly suggested that elevated plasma levels of von Willebrand Factor are not central to pathogenesis [47•].

Multiple in-vitro and animal studies have suggested the importance of cytokines and chemokines. An imbalance of pro-inflammatory and anti-inflammatory mediators may be central to pathogenesis. Interleukin-6/interleukin-10 and interleukin-6/interleukin-1 receptor antagonist ratios relate to severity of renal disease [48]. Granulocyte-colony stimulating factor is increased in HUS while neutrophil chemotactic activity is not easily interpretable (interleukin-8 is increased but epithelial cell-derived neutrophil-activiting protein-78 decreased in HUS) [49].

Recent prospective studies in children favor the concept that HUS is a prothrombotic disturbance in which a limited form of disseminated intravascular coagulation occurs [50•]. There is a gradient of coagulation abnormalities that occur in children with Stx-associated colitis with abnormalities found even in those who do not develop HUS. Those who developed HUS had higher plasma concentrations of prothrombin fragment 1+2, tissue plasminogen activator, tissue plasminogen activator–plasminogen activator inhibitor, and D-dimer. Both thrombin generation and impaired fibrinolysis precede development of HUS [51••]. Despite the similarity of HUS and thrombotic thrombocytopenic purpura, the deficiency of von Willebrand Factor clearing metalloprotease that is associated with thrombotic thrombocytopenic purpura in adults is not typical of children with diarrhea-associated HUS [52,53]. Urinary levels of the angiogenic peptide basic fibroblast growth factor may be useful as predictors of severity of HUS [54].

Diagnosis

Stool culture for *E. coli* O157:H7 is essential in all patients who have bloody diarrhea, particularly those who are afebrile. Additional tests should be done on feces to look for free Stx related to non-O157 STEC [55]. Both latex agglutination and enzyme immunoassay are available to detect toxin in stool; both assays have good sensitivity and specificity [56••]. Polymerase chain reaction and other DNA based methods are currently used primarily in research laboratories [57–59]. A variety of molecular techniques is available to determine whether a given STEC strain is responsible for an outbreak of disease or linked to an animal or environmental source. Surprisingly, high amounts of toxin detected by vero cell assay are not predictive of greater risk of HUS [60•]. Although therapeutic options are limited, definitive diagnosis of an infection is useful. An infected child can be carefully observed so that HUS may be detected and managed early. Likewise, public health measures can be useful if outbreaks are recognized early and contaminated food recalled.

Treatment

There is no consensus on therapy of hemorrhagic colitis. A non-randomized inadequately controlled study was published in 2000 suggesting that antibiotics increased the risk of HUS [61]. This study was consistent with in-vitro data as well as several other uncontrolled studies suggesting that antibiotics may increase the risk of HUS in patients with hemorrhagic colitis. Agents that damage DNA or inhibit its replication induce the phages that carry Stx genes; increased gene copy numbers leads to augmented toxin production. In contrast to other antimicrobials, Azithromycin blocks phage induction and toxin production, decreases pro-inflammatory cytokine production and improves survival after toxin injection or STEC infection in mice [62••]. However, a recent metaanalysis makes it clear that the issue is still unresolved [63].

At present it is not possible to make a recommendation regarding use of antibiotics in hemorrhagic colitis. After HUS develops, conventional management of renal failure, anemia, bleeding, and intestinal injury is appropriate.

Conclusion

Shiga toxin-triggered HUS is the most common cause of potentially preventable pediatric renal failure. Because there is a time lag of 4–13 days between onset of STEC diarrhea and the development of microangiopathic hemolytic anemia, it may be possible to prevent progression to vascular endothelial injury, localized coagulopathy, hemolysis, thrombocytopenia and renal failure. At present this promise remains unfulfilled.

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