Shiga Toxin–Producing *Escherichia coli* Infection

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Large-scale outbreaks of Shiga toxin–producing *Escherichia coli* (STEC) infection have revealed the great disease-causing potential of this organism, especially among children and elderly persons. Approximately 5%–10% of people with STEC infection will develop hemolytic-uremic syndrome (HUS), ∼10% of those who develop HUS will die or have permanent renal failure, and up to 50% of those who develop HUS will develop some degree of renal impairment. Important concepts in understanding the pathogenesis and prevention of STEC-associated HUS are emerging, although no specific therapy yet exists.

Optimal management of STEC infection includes intravenous hydration, avoidance of antimotility agents and antimicrobials, and monitoring for sequelae. Antimicrobials may have a potentially harmful role, possibly by inducing intestinal production of Shiga toxin during the diarrheal phase of illness. A recent clinical trial evaluating an intraluminal Shiga toxin–binding agent to ameliorate HUS showed no improvement in outcome. Interventions to prevent HUS from developing in STEC-infected children are under investigation. Prevention of exposure to STEC remains important, and animal vaccines to prevent stool shedding of STEC among food animals are in development.

Shiga toxin-producing *Escherichia coli* (STEC) were first discovered in 1977 [1] and first associated with the clinical syndrome hemolytic-uremic syndrome (HUS) in 1983 [2]. Since then, diarrhea-associated HUS has been recognized as one of the most common causes of acute renal failure in otherwise healthy children in the United States [3]. We cannot yet prevent HUS following STEC infection or specifically treat diarrhea-associated HUS once it has developed. However, our understanding of how STEC cause disease and our ideas about prevention and treatment strategies have evolved since this organism was first linked to HUS. In this article, recent progress that has contributed to understanding the epidemiology and disease pathogenesis of STEC is reviewed, and the state of knowledge regarding treatment of STEC infection and prevention of HUS is discussed.

**UPDATE ON EPIDEMIOLOGY**

The first large, well-publicized outbreak of STEC-associated HUS in the United States involved consumption of fast-food hamburgers, but since then, this mode of exposure has diminished in importance, and the number of outbreaks linked to consumption of ground beef in private homes has increased [4]. Also, the list of transmission vehicles that can and have resulted in STEC infection has greatly expanded. Because STEC exposure typically occurs after consumption of food and water contaminated by cattle feces, these transmission vehicles include both private and municipal water sources and a variety of food products, such as unpasteurized apple cider or milk, fresh vegetables and sprouts, and salami. Perhaps less well-known is the association between STEC infection and recreational exposures. Visits to petting zoos, dairy farms, campgrounds where cattle have previously grazed, and recreational water sources have all resulted in STEC infection [5]. This mode of STEC transmission, as well as person-to-person spread, may be explained in part by the very low infectious dose (<100 organisms) of some strains; therefore, minimal exposures can result in disease.

In the United States, HUS occurs sporadically as well as in outbreaks. Therefore, testing for STEC should be performed in sporadic cases of bloody diarrhea. How should microbiology laboratories perform such testing? An optimal testing strategy that balances sensitivity and cost-effectiveness remains undefined for several reasons. Although the first epidemiologic reports that linked STEC with hemorrhagic colitis and HUS largely implicated STEC of the O157:H7 serogroup, it is now appreciated that STEC are a heterogeneous group of organisms linked by a single feature: the ability to produce Shiga toxins. Though some studies have suggested that non-O157:H7 STEC
are less likely to cause bloody diarrhea (see [6] and references therein), STEC of many different serotypes can and do cause diarrheal disease and HUS. This strain heterogeneity makes diagnostic testing problematic, because a common, inexpensive, but relatively insensitive way of screening stool samples for STEC O157:H7 takes advantage of a metabolic quirk of this one serotype: the inability to ferment sucrose. Therefore, plating on sorbitol-MacConkey (SMAC) agar will reveal white colonies in ~50% of stool samples from cases caused by STEC O157: H7, missing one-half of these cases and missing all cases due to non-O157:H7 strains. Other, non-serotype-restricted diagnostic methods have been developed, such as screening of stool specimens for the presence of Shiga toxin proteins by either ELISA or latex agglutination, enhancing sensitivity to the 80%-90% range, although at significantly increased expense. PCR-based methods for detecting Shiga toxin genes in stool samples are in the research phase. In the United States, where it is estimated that 20%-50% of STEC infections are caused by non-O157:H7 serotypes [7, 8] (D. W. K. Acheson, personal communication), enhanced diagnostic testing to detect all STEC infections may not be cost-effective until there are clear-cut HUS therapeutic interventions useful in the diarrheal phase. Because of these considerations, the infectious disease practitioner should be aware of the type and sensitivity of tests performed by the local diagnostic laboratory, especially if antimicrobial therapy is being considered (see Old and New Clinical Insights).

FROM GUT TO KIDNEY: STEC, SHIGA TOXINS, AND HUS

To cause disease, STEC must be ingested, survive the acidic environment of the upper gastrointestinal (GI) tract, and colonize the lower GI tract. The mechanism by which colonization of the lower GI tract occurs is best described for STEC that contain the “locus of enterocyte effacement” pathogenicity island, or “LEE region” [9]. Most of the STEC associated with outbreaks, including E. coli serotypes O157, O26, and O111, harbor this pathogenicity island. First described in enteropathogenic E. coli, the LEE region encodes genes involved in stimulating intestinal epithelial cells to build a pedestal on the cell surface to which the bacteria intimately adhere, called the attaching and effacing lesion. In contrast, how STEC that do not contain the LEE region mediate colonization is not well understood.

The principal virulence factors associated with the severe sequelae of STEC infection are the Shiga toxins. The Shiga toxin family consists of a number of structurally and functionally related protein toxins (reviewed in [10]). The prototype of the family, Shiga toxin, is elaborated by Shigella dysenteriae type 1. The Shiga toxins associated with E. coli are designated by a number or number/letter combination following the name Shiga toxin (e.g., Shiga toxin 1). Shiga toxin 1 is almost identical to Shiga toxin from S. dysenteriae type 1, differing by a single amino acid. Shiga toxin 2 is ~50% homologous with Shiga toxin 1 at the protein level and is immunologically distinct. There are a number of variants of Shiga toxin 2 which may be more virulent to humans, such as Stx2c, and certain forms of Stx2d which can be activated by an elastase present in human mucus [6, 10]. In contrast, Shiga toxin 2e is primarily associated with animal disease. STEC associated with human disease can make Shiga toxin 1, Shiga toxin 2, or both. Production of Shiga toxin 2 is associated with increased risk of HUS.

Because STEC are noninvasive, it is thought that Shiga toxins must be absorbed from the intestine to cause disease. How this occurs during STEC infection is unknown. However, humans with STEC infection frequently are found to have fecal leukocytes [11]. The process of neutrophils migrating from the systemic circulation across the intestinal epithelium to the gut lumen can cause transient epithelial barrier damage, allowing systemic uptake of gut luminal contents. Recently, STEC-induced neutrophil migration has been shown to enhance Shiga toxin uptake across intestinal epithelium in vitro [12], suggesting that inflammation occurring within the host GI tract during STEC infection may promote systemic Shiga toxin uptake. Lipopolysaccharide is also absorbed from the gut during STEC infection, and may play a role in pathogenesis of HUS [7, 13].

Shiga toxins are AB5 toxins consisting of an enzymatically active A subunit covalently associated with a pantameric B subunit that mediates binding to host cells. Shiga toxins bind to host cells that bear appropriate receptors, which are neutral glycolipids bearing a terminal galactose-α1,4-galactose moiety. The principal receptor for both Shiga toxin 1 and Shiga toxin 2 is globotriaosylceramide, orGb3, but there may be other important receptors. Once bound, Shiga toxins are taken up by the host cell, trafficking to the host ribosome, where the A subunit cleaves a specific adenine from the 28S rRNA, irreversibly inhibiting host cell protein synthesis. Classically, these ribosome-inactivating toxins were thought to mediate their effects on host cells by preventing synthesis of critical host proteins needed by the cell to survive and/or properly function. Initial investigations on cytotoxicity undertaken in the 1960s and 1970s revealed that Shiga toxins were cytotoxic to some cells but not others and linked this susceptibility to appropriate surface receptor expression. An appreciation of the exquisite sensitivity of some microvascular endothelial cells to Shiga toxins’ cytotoxic effects resulted in the hypothesis that Shiga toxins directly initiated the classic HUS lesions: swelling of the glomerular endothelial cell, with detachment from the basement membrane, and the subsequent deposition of fibrin-platelet thrombi in the kidney microvasculature.
Recently, in an effort to understand how HUS is initiated after STEC infection, investigators have focused on the Shiga toxin-host cells interactions that may occur as the toxin travels from gut lumen to target organs. Such studies have assessed Shiga toxin effects on a variety of host cells, such as gut epithelium, monocytes and macrophages, and renal tubular cells, as well as endothelial cells [10, 14]. Although beyond the scope of this review, as a result of these investigations, 2 alternative roles for Shiga toxins in HUS pathogenesis have emerged: (1) as a stimulus of proinflammatory cytokine release by a number of different host cell types, including endothelium; and (2) as a proapoptotic stimulus. Whether these Shiga toxin effects contribute to HUS pathogenesis is unknown, but proinflammatory cytokine expression by the host may act to upregulate Shiga toxin receptor expression on the microvascular endothelium of target organs, thus increasing the risk of HUS (discussed in [3, 7]). The mechanism(s) by which Shiga toxins induce proinflammatory cytokine expression and apoptosis are an area of active study. Unfortunately, the lack of a small animal model accurately reproducing human disease has hampered definition of the critical pathophysiologic events that occur between the diarrheal phase of STEC infection and the development of HUS (reviewed in [7]).

OLD AND NEW CLINICAL INSIGHTS

Although STEC infection may be asymptomatic, it typically begins with a watery diarrhea that is frequently associated with abdominal pain and occasionally with nausea and vomiting. Fever is not a prominent feature, distinguishing this illness from inflammatory colitis that occurs as a result of infection with organisms such as Salmonella. The watery diarrhea may or may not progress to bloody diarrhea (hemorrhagic colitis) within 1–2 days. In its most severe form, STEC-associated diarrhea can be difficult to distinguish clinically from inflammatory bowel disease. STEC infection that progresses to HUS tends to be asymptomatic, and there is a bimodal distribution in susceptibility to HUS, with children and elderly persons being at highest risk. This complication tends to occur within 2–14 days after the onset of diarrhea. Nonrenal complications of STEC infection include ischemic colitis with colonic stenosis and, rarely, persistent pancreatitis with diabetes.

There is tremendous interstudy variability regarding progression of STEC infection to HUS, ranging from 1% to >20%. Part of this discrepancy may be the result of differences in patient classification; using strict criteria, the risk of HUS following STEC infection can be estimated to be 5%–8% (reviewed in [15]). From individual studies, some clinical markers have been associated with the progression to HUS following STEC infection, including fever, leukocytosis, and elevated C-reactive protein level (reviewed in [15]). At present, however, there is no reliable way to predict who will develop HUS following STEC infection. Rarely, HUS can occur after urinary tract infection with Shiga toxin-producing organisms.

For clinicians, one of the most frustrating aspects of managing STEC infection has been lack of known effective treatment strategies that diminish risk of progression to HUS. Recent data suggest that the amount of parenteral hydration received before the development of HUS—in particular, the salt component of the hydration—is critical in preventing anuric HUS and the need for dialysis (P. I. Tarr, personal communication). This simple maneuver may represent the first known beneficial intervention to prevent the need for dialysis in STEC-infected children who develop HUS.

There has been significant controversy surrounding antimicrobial treatment of STEC infection. Treatment of STEC infection in the diarrhea phase with certain antimicrobials (specifically, DNA-damaging antibacterials) has been associated with HUS (reviewed in [16]). However, use of other antimicrobials has been associated with protection from HUS (reviewed in [17]).

The reasons for this clinical observation may be explained by understanding how STEC carry the genes that encode Shiga toxins. Unlike S. dysenteriae type 1, Shiga toxins produced by STEC are carried on bacteriophages homologous to the phage lambda. These lambdoid phages can exist in 2 phases, called “lysogenic” and “lytic.” In the lysogenic phase, the bacteriophage is incorporated into the bacterial cell chromosome and undergoes replication with the host DNA. In the lytic phase, the bacteriophage excises itself from the host chromosome and replicates independently, making many copies of itself and eventually bursting forth from the host cell.

Induction of this lytic phase may significantly increase production of Shiga toxins in several ways: (1) by replication, thereby increasing the copy number of Shiga toxin genes; (2) by a bacteriophage-encoded transcriptional activator that increases transcription from phage-encoded genes, such as Shiga toxins; and (3) by linking Shiga toxin production with cell lysis [18]. One common signal that induces bacteriophage to enter the lytic cycle is bacterial DNA damage. In vitro, antibacterials that act by causing DNA damage, such as quinolones and trimethoprim, have been shown to dramatically increase both bacteriophage and Shiga toxin production from certain STEC strains, and these observations have been confirmed in a murine model (see [19] and references therein). In this model, STEC-infected mice treated with ciprofloxacin had higher stool levels of both bacteriophage and Shiga toxin than did control mice, and this was associated with mouse death. In contrast, the cell-wall active agent, fosfomycin, did not increase either mortality in mice or Shiga toxin in their stool samples, compared with control mice. Although we do not yet know what factors may trigger bacteriophage production in the human patient, studies
of this type provide some insight into the basic mechanism(s) by which treatment with certain antibacterials may promote HUS following STEC infection.

In 2000, a prospective study performed in the Pacific Northwest showed that children who received antibiotics during the diarrheal phase of illness had an increased risk of developing HUS [20]. Because of the small numbers of children included in this study, 95% CIs were wide-ranging, leading to some criticism of these data. However, with continued enrollment of subjects in this study, the risk remains elevated, and the 95% CIs are decreasing (P. I. Tarr, personal communication). In 2002, a meta-analysis of 9 studies in which the relationship between antibiotic use and HUS was assessed discovered no association between antimicrobial use and HUS [17]. This meta-analysis was highly criticized for many methodologic reasons, including lack of prospective data, differences in patient populations studied, and heterogeneity in type, timing, and duration of antimicrobials used. In the absence of data from a nationwide, prospective, randomized, controlled trial, antimicrobial use should probably be avoided during STEC infection, and many practitioners are still adhering to this 2001 guideline of the Infectious Diseases Society of America [16]. Other practice guidelines for management of STEC infection include intravenous hydration, avoidance of antimotility agents, and monitoring for development of thrombotic microangiopathy and renal dysfunction.

How the procoagulant cascade becomes initiated in diarrhea-associated HUS remains unknown. In 2002, an interesting study was published that assessed blood procoagulation factors and urinary renal tubular injury markers in 53 children with STEC infection during the diarrheal phase of illness, before HUS had developed [21]. Of those 53 children, 37 had uncomplicated STEC O157:H7 infection; 16 children went on to develop HUS. Comparison of STEC-infected children with healthy control subjects showed that prothrombotic abnormalities (both increases in thrombin production and fibrinolysis impairment) and markers of renal tubular injury were common, whether HUS developed or not. Furthermore, the severity of the prothrombotic abnormalities was proportional to the likelihood of developing HUS. Although a small number of patients were assessed, these data suggest that a severe, prothrombotic coagulation disturbance exists during STEC infection, supporting additional investigation into how this disturbance develops and raising the following question: if this coagulation disturbance already exists during the diarrheal phase of illness, when are intervention(s) to prevent or treat STEC-associated HUS most likely to be effective?

The long-term renal prognosis after diarrhea-associated HUS has recently been the subject of review and meta-analysis [22], incorporating data from 49 studies describing 3476 patients aged 1–22 years who have either sporadic disease or outbreak-associated disease involving a number of different food vehicles.

In this analysis, ~12% of patients had either died (9%) or developed end-stage renal disease (ESRD) (3%) by 4 years after diarrhea-associated HUS. Most of the deaths occurred during the acute phase of illness. (Because this meta-analysis includes reports from the 1960s, when life-saving peritoneal dialysis was not an established treatment modality, the percentage of deaths during the acute phase is probably an overestimation. In the modern era, ~3%–5% of patients die of HUS during the acute phase [23].)

In meta-regression, the presence of CNS symptoms was strongly associated with a worse long-term outcome, as was the need for acute dialysis. Similarly, when the duration of acute dialysis therapy exceeded 4 weeks, none of the patients achieved full renal recovery. Of the survivors, on average, 25% had some evidence of long-term renal sequelae. Because improvement in glomerular filtration rate can occur up to a year after post-diarrheal HUS, the authors suggest that patients with diarrhea-associated HUS be screened at least once for occult renal disease a year after the insult, with determination of serum creatinine level, urinalysis including dipstick for proteinuria, and determination of blood pressure. This study was limited for several reasons [3], the most important of which is that the average duration of follow-up was only 4.4 years, therefore probably underestimating the true incidence of long-term renal disease after HUS. Because progression to ESRD following HUS may occur over 15–25 years [24], a single screening for renal dysfunction 1 year after the insult may be inadequate.

HUS PREVENTION AND TREATMENT STRATEGIES

Because STEC are noninvasive, one possible tactic to prevent or ameliorate HUS involves preventing further uptake of Shiga toxin from the gut, either during the diarrheal phase or once HUS has developed. A variety of strategies aimed at preventing toxin uptake have been developed that rely on administration of a molecular decoy bearing what is thought to be the highest affinity receptor for Shiga toxins, globotriaosylceramide (Gb3). No clinical trials have yet been reported using such agents to prevent HUS in STEC-infected children. However, data from a multicenter, randomized, double-blind, placebo-controlled trial were recently reported, aimed at diminishing disease severity in children aged 6 months to 18 years who have already developed diarrhea-associated HUS [25]. This trial evaluated the effects of one such compound, a nonabsorbable silicon-based binding matrix linked to Gb3 called SYNSORB-Pk (Synsorb Biotech). Patients who received SYNSORB-Pk had a similar prevalence of death or serious extrarenal events, need for dialysis, and duration of dialysis. Although there are a number of possible explanations for this trial’s lack of efficacy, the most
pessimistic is that, once HUS has developed, there may be little additional benefit to preventing further Shiga toxin uptake, because the damage has already been done.

Shiga toxin–neutralizing monoclonal antibodies and Shiga toxin absorbers have been effective in a number of animal studies. A clinical trial designed to test the effectiveness of passive administration of neutralizing anti–Shiga toxin antibody in prevention of HUS has been initiated at McGill University in Montreal, Canada. Children who have bloody diarrhea for <72 h and whose stool samples are positive for E. coli O157: H7 will be eligible to participate in this study, which is testing the hypothesis that early administration of antibody will inactivate toxin in the bloodstream, this preventing end-organ damage. Results from this study will be of great interest.

What can be done once HUS has developed? It has been suggested that blocking downstream events in the pathogenic cascade, such as Shiga toxin–induced production of cytokines, may be beneficial [3]. Although this strategy has not yet been tested in subhuman primates, Siegler et al. [13] recently developed a baboon model of Shiga toxin–induced HUS that faithfully reproduces human disease that will undoubtedly facilitate further research in this area.

**STEC PREVENTION STRATEGIES**

Because STEC-associated HUS is a relatively rare disease, widespread vaccination of human populations at risk may not be cost-effective. One possible vaccination strategy involves immunization of cattle to reduce the prevalence of STEC colonization, thereby reducing the likelihood of STEC entering the food chain. A clinical trial was recently conducted in feedlot cattle that tested the efficacy of immunization against secreted E. coli O157:H7 proteins in reducing prevalence of E. coli O157: H7 shedding [26]. The average proportion of cattle shedding E. coli O157:H7 in vaccine-treated pens (8.8%) was significantly lower (P < 0.05) than in nonvaccinated pens (21.3%). Ultimately, expansion of vaccine efforts of this type may prevent colonization and shedding of multiple pathogenic STEC serotypes, thereby diminishing food contamination before it reaches consumers.

A recent example demonstrates how widespread this contamination can be, as well as how routine microbiologic testing performed by government regulatory agencies can be beneficial. In June 2002, the ConAgra Beef Company issued a nationwide recall of ground beef products when routine microbiologic testing performed by the US Department of Agriculture revealed STEC contamination [27]. If widely consumed, the recalled meat could have caused an epidemic of massive proportions: contaminated meat consumed before the national recall was implicated in a small, contained epidemic of Shiga toxin–producing E. coli infection that was clustered in Colorado and that involved residents of 6 other states. Even in this small epidemic, >5 cases of HUS occurred.

Clearly, the food safety practice of cooking ground beef to 72°C (160°F), or until meat is no longer pink, has been widely adopted by the fast-food industry, contributing to the decrease in STEC infections traced to this vehicle. This practice is perhaps not as consistently followed by the private consumer who enjoys rare hamburger, although it is possible that ground beef irradiation may allow these consumers to eat rare hamburger with impunity. Although it is unreasonable to advocate avoidance of fresh fruits and vegetables that may be contaminated at the farm level as a prevention measure, it is practical to educate consumers about food-safety practices that would limit contamination of uncooked foods within the consumer kitchen. These measures include using separate utensils and cutting surfaces to prepare raw meats and frequent hand washing. Until effective HUS prevention and treatment strategies are developed, limiting consumer exposure to STEC remains important.

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**References**

9. Paton JC, Paton AW. Pathogenesis and diagnosis of Shiga toxin-