EXPERT REVIEWS

Hemolytic uremic syndrome: pathogenesis and update of interventions

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The typical form of hemolytic uremic syndrome (HUS) is the major complication of Shiga toxin-producing *Escherichia coli* infections. HUS is a critical health problem in Argentina since it is the main cause of acute renal failure in children and the second cause of chronic renal failure, accounting for 20% of renal transplants in children and adolescents in Argentina. Despite extensive research in the field, the mainstay of treatment for patients with HUS is supportive therapy, and there are no specific therapies preventing or ameliorating the disease course. In this review, we present the current knowledge about pathogenic mechanisms and discuss traditional and innovative therapeutic approaches, with special focus in Argentinean contribution. The hope that a better understanding of transmission dynamics and pathogenesis of this disease will produce better therapies to prevent the acute mortality and the long-term morbidity of HUS is the driving force for intensified research.

Keywords: *Escherichia coli* O157:H7 • hemolytic uremic syndrome • inflammation • Shiga toxin • treatment

General concepts

The typical form of hemolytic uremic syndrome (HUS) is caused by particular serotypes of enterohemorrhagic *Escherichia coli* (EHEC) that produce Shiga toxin (Stx), and are known as STEC (Stx-producing *E. coli*). Full development of HUS is characterized by thrombocytopenia, hemolytic anemia and acute renal failure, which appear a few days after the infection. Wagner may have been the first to report a case of HUS [1], and Gasser *et al.* described the features of HUS in 1955 [2]. In Argentina, the group of Gianantonio in 1964 published the first complete description of the clinical features and evolution in more than 50 cases of HUS [3] and most importantly, they proposed peritoneal dialysis as treatment in the acute period [4]. Later, in 1983, Karmali *et al.* described the association between *E. coli*, Stx and HUS [5].

Hemolytic uremic syndrome is the main cause of acute renal failure in children in Argentina, and a high percentage of children with HUS (20–30%) have long-term permanent renal sequelae, making this disease the second cause of chronic renal failure and accounting for 20% of renal transplants in children and adolescents in Argentina [6,7]. HUS is not exclusively restricted to the kidney. Many patients during the acute period experience neurological symptoms (20%, including lethargy, seizures, headache, convulsions and coma), hypertension (30–50%), pancreatic insufficiency (8–10%) and/or gastrointestinal complications (severe colitis and rectal prolapse) [6,8]. In this sense, severe cases of HUS, and usually death, are associated with central nervous system involvement. Other extra renal long-term complications can also be observed, mainly hypertension, but also neurological sequelae (moderate and severe disability) and insulin-dependent diabetes mellitus have been reported [7,9,10].

Infection begins with ingestion of contaminated food or water. Acid-resistance mechanisms of STEC facilitate their survival through the low pH of the stomach [11]. The bacteria pass through the small intestine, and virulence genes are turned on by environmental signals in the colon [12]. Adherence to enterocytes of the colon is produced by a characteristic mechanism called attaching and effacing (A/E) lesions, in which bacteria intimately attach to the intestinal epithelial cells and rearrange the cytoskeletal actin underneath, causing diarrhea and intestinal inflammation [13]. Local damage in colonic blood vessels induced by Stx results

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in bloody diarrhea. Recent evidence indicates that macropinocytosis might be responsible for toxin uptake and transcytosis by globotriosylceramide (Gb_3)-free intestinal epithelial cells [14]. If sufficient Stx is absorbed into the circulation, vascular endothelial sites rich in the toxin receptor are damaged, leading to impaired function.

Pathophysiology

Gianantonio described in1973: 'The pathologic renal findings in the acute stage of HUS show that most of the lesions are expressions of intravascular clotting' [15]. Renal histology reveals thickening of the capillary wall and swelling of endothelial cells within the glomerulus, with reduction of glomerular capillaries [16]. Nowadays, it is well known that the microvascular endothelial damage is a central pathogenic process underlying the development of HUS. However, human glomerular endothelial cells *in vitro* are not very susceptible to Stx toxic effects unless pretreated with inflammatory mediators [17]. Histology also shows that renal tubular cells can be necrotic. It has been demonstrated that Stx directly affects renal tubular cells [18] and triggers the activation of the coagulation and inflammatory systems [19,20], which, in turn, sensitize endothelial glomerular cells to Stx toxic effects [17]. Regardless of which cell type is the first target of Stx, the extent of glomerular injury will be determinant in long-term outcome. In fact, affected glomeruli will become fibrotic reducing the number of functional nephrons.

Shiga toxin

Stx is the main virulence factor in STEC infections. The *stx* gene is encoded in lysogenic bacteriophages contained by the bacteria. The production of Stx is linked to the replication cycle of the Stx phage, and the bacteria must be lysed in order to release the toxin [21]. Two major subfamilies of Stx exist: Stx1 and Stx2. In addition, each subfamily consists of different variants. Members included in the Stx1 subfamily are Stx1, Stx1c [22] and Stx1d [23], whereas Stx2, Stx2c [24], Stx2d [24], $Stx2d_{activable}$ [25], Stx2e [26] and Stx2f [27] belong to the Stx2 group. Although all these variants have been isolated from patients, not all of them are associated with severe disease. In this sense, it has been reported that bacteria producing only Stx2 are more pathogenic than those producing Stx1 alone and those producing Stx1 with Stx2. Those producing Stx2, Stx2c and $Stx2d_{activable}$ have been associated with more severe disease, such as hemorrhagic colitis and HUS, whereas the other members of Stx1 or Stx2 subfamilies were associated with uncomplicated diarrhea and asymptomatic infections [28]. All Stx members possess one catalytically active A subunit and five B subunits that mediate the binding to Stx receptor, Gb_3. Stx induces different receptor-mediated biological effects depending on the cell type, which is related to the organization of Gb_3 in the membrane and directs the fate of the toxin inside the cell. Stx cytotoxicity is dependent on Gb_3 presentation within detergent-resistant membrane (DRM) domains or 'lipid rafts' [29,30]. Internalization of Stx through Gb_3 within plasma membrane DRM results in a retrograde transport ending in the cytosol, and the interaction of the A subunit with the 28S rRNA leads to inhibition of protein synthesis and, eventually, cell death [31]. By contrast, non-DRM plasma membrane Gb_3 mediates binding and subsequent traffic to lysosomes for Stx degradation [32,33]. In addition, the dose is also a determinant in Stx-mediated effects. In fact, sublethal amounts of Stx have only minor effects on overall protein synthesis but have dramatic effects on gene regulation, causing increased expression of hallmark proadhesive, prothrombotic and inflammatory genes in, for example, endothelial cells [34–37].

Additional factors

Hemolytic uremic syndrome is multifactorial in etiology involving complex interactions between bacterial and host factors. Although in the USA and Europe, 95% of HUS cases are associated with a STEC infection [38], in Argentina this association is confirmed only in the 60% of HUS cases, by at least one of the diagnosis criteria used [39]. This discrepant result may be attributed to deficiencies in local systems of diarrhea surveillance. On the other hand, not all STEC infections result in HUS. In fact, it may result in no disease, watery diarrhea, bloody diarrhea or HUS. Only 5–10% of infected children develop HUS [40]. The initial bacterial inoculum, the amount and type of Stx produced by the bacteria ingested, additional bacterial virulence factors, and the specific characteristic of activation of the thrombotic and inflammatory responses of the host are some of the factors that may determine the outcome of a STEC infection.

Additional factors from the bacteria

Besides Stx, putative virulence factors of STEC including adhesins, other toxins and proteases [39,41,42], are required to develop disease. The precise role of some of these factors in STEC disease remains to be fully established. It is possible that the degree of adhesion is correlated with the ability to cause disease. Whereas adhesins are probably implicated in the first contact to cells, intimate adhesion is mediated by the proteins encoded in the locus of enterocyte effacement (LEE), which participate in the formation of the A/E lesion. These include a type III secretion system, the *E. coli*-secreted proteins (Esp) and the surface protein intimin [43]. It is important to point out that several LEE-positive STEC strains are not associated with HUS, and that the type III secretion system also secretes many other effector molecules encoded outside the LEE, which are referred to as non-LEE-encoded effectors [44]. At least three of these non-LEE-encoded effectors have been linked to non-O157 STEC that cause HUS [45].

The intimin gene *eae* has been identified as a risk factor for HUS, as the vast majority of strains implicated in HUS are intimin positive [46]. However, recently it has been shown that highly pathogenic strains producing $Stx2d_{activable}$ are intimin negative [47].

Among non-Stx toxins, the plasmid-encoded enterohemolysin (Ehx) is a pore-forming cytolysin produced by both *eae*-positive and *eae*-negative STEC, but the frequency is higher among *eae*-positive STEC [48]. Ehx could contribute to disease by its membrane damaging effect on a wide variety of cell types, including

erythrocytes, its ability to induce production of proinflammatory cytokines, or both [49]. Although Ehx has been found in many STEC serotypes that are commonly associated with diarrheal disease and HUS [50–52], it is not clear how this toxin could enter the circulation to cause systemic damage.

Subtilase cytotoxin is a toxin with an A1:B5 structure, detected in a high percentage of STEC strains, mainly in LEE-negative strains [53]. In contrast to Stx, it was recently described that glycosphingolipids are not pivotal receptors for this toxin *in vivo* [54]. It has been shown to transiently inhibit protein synthesis and cause apoptosis of the Stx-sensitive Vero cell line [55,56]. In addition, intraperitoneal injection of subtilase cytotoxin to mice causes death, microangiopathic hemolytic anemia, thrombocytopenia and renal impairment, characteristic features of Stx-induced HUS [53,57].

Bielaszewska *et al.* described another novel toxin, the cytolethal distending toxin (CDT-V), present in 87 and 6% of O157:H$^-$ and O157:H7 strains, respectively [58]. CDT-V has also been found in non-O157 STEC strains, all of which were *eae* negative [59]. This toxin directly injures endothelial cells leading to their death, and may thus contribute to the pathogenesis of HUS [59].

Several proteases (EspA, EspP, EspJ, EspI and StcE) are produced by STEC, and their activities suggest a putative role in the pathogenesis of the disease [43,60,61].

Lipopolysaccharide (LPS) is a major product of the Gram-negative bacteria that synergizes Stx-induced toxicity. Although Louise *et al.* suggested that Stx may enhance the procoagulant effect of LPS [62], *in vivo* studies have demonstrated that toxicity of Stx is potentiated by LPS [63–65]. In particular, LPS, chemokines and cytokines released by inflammatory cells or injured cells upregulate the expression of Stx receptors and sensitize microvascular endothelial cells (glomerular and others) to Stx-induced injury [17,66–68].

In summary, although Stx-induced endothelial injury is the primary pathogenic event, multiple bacterial components may contribute to the whole pathogenic mechanism.

Factors from the host

Several authors have reported the importance of the inflammatory and thrombotic responses in the development of HUS. Initially, STEC colonization induces acute colonic inflammation. In this regard, the infiltration of the gut and the presence of leukocytes in feces are seen in many STEC-infected patients. Several pathogenic factors of STEC have been demonstrated to induce the expression of proinflammatory chemokines in epithelial cells, which was accompanied by an influx of polymorphonuclear leukocytes (PMNs) [18,69]. Transmigration of PMNs from the basolateral to apical area of an intestinal epithelial cell line significantly increased the movement of Stx1 and Stx2 in the opposite direction [70]. In addition, PMN recruitment in the intestine may also increase the risk of HUS by inducing the Stx2 prophage *in vivo* and augmenting Stx2 production, mainly through the production of H_2O_2 [71].

Patients also evidence a marked inflammatory response as demonstrated by systemically (blood) and locally (urine) increased levels of various inflammatory mediators, including interleukins, chemokines, soluble adhesion molecules, growth factors and acute-phase response proteins. In addition, they also show markers of endothelial injury, activation of the coagulation cascade and inhibition of fibrinolysis [72]. It has been suggested that the degree of the prothrombotic activation early in infection could be decisive in the course of the disease [73].

The activation of PMNs is evidenced by a high peripheral blood PMN count at presentation, which has been correlated with a poor prognosis, and increased levels of serum elastase and IL-8. In addition, PMNs from HUS patients show increased adhesive capacity *in vitro* and reduction in their granule content as demonstrated by ultrastructural, phenotypic and functional studies [74–76]. In this sense, the severity of renal impairment has been correlated with the degree of PMN degranulation [77]. Platelet activation has been suggested by several evidences. Thrombocytopenia is a main feature of HUS, and is caused by consumption of platelets, probably after activation and aggregation. Platelets from HUS patients showed impaired aggregating responses and reduced β-thromboglobulin (β-TG) content [78]. Platelet-derived products such as β-TG, platelet factor 4 and soluble P-selectin were found to be elevated during acute HUS [79,80]. Furthermore, changes in platelet ultramorphology and increased platelet-derived microvesicles were found in these patients, indicating platelet activation [81]. The activation of both PMNs and platelets will potentiate the inflammatory process and may enhance the primary Stx-induced endothelial damage.

Activated monocytes may also contribute to Stx toxic effects by the secretion of several chemokines and cytokines (TNF-α, IL-1β, IL-8, RANTES and tissue factor), which increase endothelial susceptibility to Stx. An increased percentage of CD16$^+$ monocytes have been reported in peripheral monocytes from HUS patients [82]. These CD16$^+$ monocytes are considered to represent an activated and more mature subset with characteristics that resemble macrophages and dendritic cells [83,84]. Fractalkine (FKN; CX_3CL1) is a transmembrane chemokine present on endothelial and epithelial cells, whereas the FKN receptor (CX_3CR1) is expressed on monocytes, among other cells. Under conditions of physiologic flow, FKN mediates adhesion of monocytes, and is upregulated upon inflammation. A disappearance of CX_3CR1^+ monocytes has been reported in HUS patients, and this correlated with the severity of renal failure [85]. The fact that CX_3CR1^+ leukocytes were observed in renal biopsies from patients with HUS suggest that the interaction of CX_3CR1^+ cells with FKN present on activated endothelial cells may contribute to renal injury in HUS.

Epidemiology

Globally, 70–80% of HUS cases have been attributed to epidemic outbreaks with O157:H7/H$^-$ strains. However, other serotypes are also capable of causing human disease [86]. In Argentina, where HUS is endemic, the dominant STEC serotype isolated from HUS patients is O157:H7, followed by O145:NM, O26:H11, O103:H21, O174:H21, O8:H19 and O145:H25 serotypes, in descending order [87]. It is important to point out that non-O157 STEC is much harder to detect microbiologically, and for this reason the proportion of HUS cases caused by O157 STEC may be an overestimate.

The incidence of HUS varies according to the country. Argentina shows the highest incidence worldwide with 12–14 cases per 100,000 children under 5 years of age per year, and more than 400 new cases per year [87]. This rate is ten-times higher than in other industrialized countries [88].

In Argentina, as in the rest of the world, healthy cattle are the principal and natural reservoir of *E. coli* O157:H7 [89]. STEC strains have been recovered from fecal samples of 39% of healthy animals in a recent Argentine survey [90]. STEC is transmitted to humans through contaminated food and water [91–93]. Other routes of transmittion are associated to prolonged fecal shedding of STEC among children attending daycare centers [94] and direct contact with infected individuals [95] or animals [96].

Examples of reported sources include undercooked ground beef, private or municipal water sources and other food products, such as unpasteurized apple cider or milk, fresh vegetables, sprouts and salami [97]. Visits to petting zoos, dairy farms, camping grounds where cattle have previously grazed, and recreational water sources have all been shown as risk factors for infection. In Argentina, person-to-person transmission has been proposed as an important route of infection supporting endemic behavior and family outbreaks [98]. A very low infectious dose (100–500 organisms) of this microorganism is required to become infected, accounting for the ability to cause severe and epidemic disease [44].

Treatment

Although there is extensive research in the field, the mainstay of treatment for patients with HUS is supportive therapy that generally includes control of fluid and electrolyte imbalance, use of dialysis if required, control of hypertension and blood transfusion as required.

Treatments during pre-HUS interval

Since vascular occlusion could partly underlie renal insufficiency during HUS, Ake *et al.* recommended parenteral volume expansion of children with bloody diarrhea associated to *E. coli* O157:H7 infection and before HUS develops [99]. Volume expansion might attenuate renal failure and also mitigate the nephrotoxicities of filtered urate and hemoglobin. They have encouraged hospitalization and intravenous administration of isotonic fluids, based on the fact that salt loading protects against nonthrombotic nephrotoxicity and hyponatremia, a potential complication of hypotonic maintenance fluids. However, more randomized studies evaluating this point would be necessary to generalize this early treatment, and most important, children who receive intravenous volume expansion need hospitalization and careful monitoring. Moreover, this recommendation makes essential a very rapid assessment of stools for *E. coli* O157:H7 detection by microbiologists and the immediate report of presumptive positives, since this treatment must be given not later than 2 days after the beginning of *E. coli* O157:H7-associated diarrhea.

The use of antimotility drugs or opioid narcotics have been discouraged in children with acute diarrhea, because of their association with complications of *E. coli* O157:H7 infection and with the prolongation of symptoms. Thus, antidiarrheal agents are usually avoided, as it is thought that this contributes to retention of Stx within the colon, which could enhance absorption of the toxin [40,100–102].

Antibiotic treatment & potential preventive agents

There is a long history of the discussion of antibiotic treatment for EHEC-induced diarrhea. An extensive analysis of the *E. coli* O157:H7 outbreak in Sakai City, Japan, suggested that treatment with fosfomycin was associated with a significantly decreased risk of HUS. However, this study has several drawbacks. Fosfomycin was compared only with other antibiotics, not with the absence of antibiotic-treatment [103,104], the benefit for antibiotic treatment was only restricted to patients that received the drug on day 2 of their illness, and this study was limited to children infected with the same strain, as is the case in analyses of outbreaks. On the other hand, most occidental researchers currently believe that antibiotics should not be administered to patients with definite or possible enteric STEC infection [105]. Several studies demonstrated that children with hemorrhagic colitis associated with EHEC who received antibiotic therapy were more likely to develop HUS compared with children who did not receive antibiotic therapy [102,106,107]. Antibiotics increase the risk of the HUS by enhancing phage induction and subsequent *stx* gene expression, and by increasing Stx release after induced bacteria lysis [108].

A recent prospective cohort study conducted by a network of 47 laboratories from different states in the USA confirmed that administering sulfa-containing antibiotics to children infected with *E. coli* O157:H7 increases their risk of developing HUS [107], and indicate that β-lactam antibiotics are associated with a similar degree of risk.

We retrospectively reviewed the outcome of 54 patients who received trimetoprim/sulphamethoxazole out of 641 HUS patients admitted to our nephrology unit from 1970 to 1990, and compared their long-term follow-up with those who had not received antibiotics. There was no significant difference between the two groups [Ramón A Exeni, Personal Observation].

In conclusion, during the diarrhea phase, antibiotic treatment should be avoided, as beneficial effects regarding initiation of HUS cannot be deduced from recent studies.

Michael *et al.* have recently reviewed seven randomized, controlled trials for interventions to evaluate their effectiveness for relevant clinical outcomes [109]. They reported that, since the first randomized trial conducted in Argentina [110], several anticoagulation therapies were assayed including heparin alone, heparin and urokinase, or heparin and dypyridamole [109]. In all these trials, the treatment and control groups received supportive therapy. Although there was significant heterogeneity between studies, there was no significant difference between groups for any of the primary or secondary outcomes, including mortality, neurological events and proteinuria or hypertension at the last follow-up. However, the incidence of bleeding (adverse effect) was significantly greater in the group that received anticoagulation therapy compared with supportive therapy alone.

Another Argentinean group conducted a trial (94 patients) comparing steroids with placebo, and there was no significant difference between the two groups for any of the outcome measures of interest [111].

Based on a previous report showing inadequate erythropoietin synthesis in children with HUS [112], it has been recently proposed early administration of erythropoietin. This new treatment reduced the need for red blood cell transfusion in HUS children [113]. The results of this pilot study will have to be confirmed in a larger multicenter trial.

Some trials have used fresh frozen plasma infusion, given no additional benefits compared with supportive therapy [114,115].

A nonconventional treatment for a very severe group of HUS patients, with clinical hemodynamic parameters of septic shock and neurological dysfunction at onset, has been proposed by Valles *et al.* [116]. The protocol includes fresh frozen plasma infusions, methylprednisolone pulses (10 mg/kg/day) for three consecutive days and plasma exchange for 5 days, starting after admission to the intensive care unit. Since nine out of 12 patients survived, compared with five deaths among a historical group of six children with the same severe form, the authors suggest that early introduction of this protocol could benefit HUS patients with hemodynamic instability and neurological dysfunction at onset.

Dialysis

The majority of children with HUS develop some degree of renal insufficiency. Gianantonio innovatively established peritoneal dialysis to manage the acute renal failure in HUS, reducing the mortality from 50 to 5% [4]. Approximately two-thirds of children with HUS will require dialysis therapy, and approximately a third will have milder renal involvement without the need for dialysis therapy [117]. Peritoneal dialysis and hemodialysis modes have been used. In most centers in Argentina, as well as in other countries, peritoneal dialysis is the preferential choice especially when patients are below 1–2 years of age [118,119]. It has been argued that peritoneal dialysis may have a higher risk of peritonitis in patients with bloody diarrhea. However, this has not yet been reported, and there are no randomized, control trials comparing the effectiveness of different types of dialysis (peritoneal dialysis, hemodialysis or continuous venous hemofiltration) in patients with acute renal failure caused by HUS.

Experimental strategies in patients with HUS

One of the first specific therapeutic approaches that raise expectation among pediatric nephrologists was the idea of binding the released Stx in the gut via amorphic compounds. In this regard, a diatomaceous silicon diamide compound linked to an oligosaccharide chain (Synsorb® Pk) was shown to avidly bind and neutralize Stx. However, the Synsorb Pk was not found to be beneficial in preventing extrarenal complications or decreasing the duration of dialysis in children with new-onset HUS [120]. Thereafter, Gb_3 polymers have been developed and showed to entrap Stx in the gut and prevent EHEC toxicity in mice [121]. Under a similar reasoning, Paton *et al.* engineered a recombinant bacterium displaying a Stx receptor mimic on its surface, which neutralized Stx with very high efficiency, and completely protected STEC-challenged mice when administered three-times daily [122]. This approach is still under investigation. The major drawback of these proposals is that barely traces of Stx that reach circulation would be enough to induce HUS, although these compounds showed a high affinity for Stx and can neutralize significant amounts of Stx in the intestine.

Taking this consideration into account, analogs of the Gb_3 receptor for administration systemically are being developed. Among them, Starfish® is a new compound shown to bind Stx 1000-times more efficiently than Synsorb Pk and has the potential to be administered intravenously. Starfish has been shown to protect mice against a lethal dose of Stx1 but not Stx2, whereas a modified version of Starfish, called Daisy®, protected mice against lethal doses of Stx1 and Stx2 [123]. Alternatively, Nishikawa *et al.* identified a structure with potent Stx-neutralizing activity in the circulation. This compound, named SUPER TWIGS, is formed by 18 trisaccharides of Gb_3, with the capacity of forming complexes with Stx in circulation that enables efficient uptake and degradation of Stx by macrophages [124]. The same authors have described a peptide-based Stx2 inhibitor that has remarkable therapeutic potency and appears to function by inducing aberrant cellular transport and degradation of Stx2 [125]. In addition, there are new therapeutic strategies, including pharmacologic inhibitors of Gb_3 [126], and several anti-Stx antibodies against the different subunits of Stx, from both Stx1 or Stx2 variants, and humanized monoclonal antibodies against Stx. The antibodies are intended to neutralize circulating Stx1 and Stx2, thereby treating the pathogenic agent of the disease and preventing serious complications such as bloody diarrhea, destruction of red blood cells and platelets, and HUS.

The production, characterization and evaluation of a panel of human monoclonal antibodies in transgenic mice were shown to effectively protect mice and piglets against the corresponding toxin challenge [127]. However, it is important to highlight that the absence of antigenic cross-reactivity between B subunits of Stx1 and Stx2 encourage the development of Stx2 antibodies, since Stx2 or its derivatives are the most frequently HUS-associated toxins.

Moreover, monoclonal antibodies directed to the Stx2 A subunit have been proved to be equal or more protective than those directed to the B subunit [128,129]. Although the mechanisms involved are still a matter of discussion, it has been recently reported that anti-A subunit antibodies interfere with retrograde transport of the toxin, preventing toxin-mediated cell death, and may interact with Stx2 when still bound to membrane receptors [130]. In addition, antibodies directed to the Stx2 A subunit as opposed to those directed against the B subunit, have broad-spectrum activity that includes other Stx2 variants, such as Stx2c [128]. Some of these antibodies have recently been approved with the orphan drug status by the US FDA and by the EMEA, for preventing HUS in a dose-escalating, Phase I clinical trial of STEC-infected pediatric patients.

Although therapeutic strategies generally attempt to neutralize bacterial virulence factors, some new proposals have challenged the blocking of host factors that contribute to the pathogenic process. In particular, it has recently been postulated that during typical HUS, the local activation of complement via the alternative pathway by Stx may also play a pathogenic role and then, inhibition of complement cascade may be beneficial [131]. On the other hand, since the inflammatory reaction is important in HUS evolution, TNF-α and cytokines might also be candidates as targets for therapeutic inhibition [28].

STEC diagnosis

One of the limitations for all these new therapeutic approaches is that the window for application is very small. In particular, the diagnosis of STEC infection should be made within 2 days after the initiation of diarrhea, a challenge that cannot be achieved routinely, at least in Argentina. Different reasons may contribute to the inability to identify STEC-infected children as early and accurate as should be done. STEC bacteria not always can be isolated and characterized from stools of diarrheic children, even in children where bloody diarrhea progressed to typical HUS [39]. In addition, the huge number of childhood diarrheas during warm seasons makes critical a sustained community education regarding the need to seek immediate medical attention in the event of bloody diarrhea and highlight the importance of microbiologic evaluation of all of them. An additional limitation is that not all healthy centers from undeveloped regions have the capability to characterize the isolated bacteria by molecular techniques such as PCR. Other criterion for STEC infection is the presence of Stx in stools. However, it has recently been reported that free fecal Stx is detected only in a low percentage of STEC-infected children and, noticeably, the lowest percentage of detection was in the group of children who evolved to HUS [132]. These findings raise the possibility that Stx was produced in greater quantities earlier in the illness and absorbed from the small bowel and not from colonic contents. The third criterion for STEC infection has been the detection of anti-Stx1/Stx2 antibodies in serum. However, the kinetic of antibody production not always allow the detection of them early in the course of infection. Therefore, community education, improvement of local surveillance systems, development of simple and economic diagnostic tools, and identification of predictors for bad evolution are imperative to allow application of new therapeutic tools in those children at high risk of HUS development.

Interventions to avoid STEC transmission

Since cattle and their products are associated with the majority of cases of *E. coli* O157:H7 infection in humans, they represent an attractive target for preslaughter intervention to reduce the risk in humans. A number of approaches are being studied to reduce levels of the organism in cattle, including animal management practices such as chlorination of water [133], modifications in animal feeding [134], the use of probiotics [135] and bacteriophage therapy [136]. Vaccinations using different immunogens are also being investigated [137]. One big potential barrier is that ranchers and feedlots may have little incentive to pay for such treatments.

Efforts to develop vaccines for people also face barriers. A Phase II trial of O157:H7 serotype LPS vaccine was conducted in 49 children between 2–5 years of age in the USA. An increase in serum IgG LPS antibodies was reported up to 6 months after vaccination [138]. However, since this vaccine does not protect against non-O157 STEC strains and outbreaks in industrialized countries are sporadic, it is difficult to test it in clinical trials. Another concern is that any proposed vaccine should be very safe, because it would be given to children and because the majority of STEC-infected children improve without any intervention.

Expert commentary & five-year view

Currently, there are no specific therapies preventing or ameliorating the disease course. Although there are new therapeutic modalities in the horizon for typical HUS, present recommended therapy is merely symptomatic and supportive. Parenteral volume expansion may counteract the effect of thrombotic processes before development of HUS and attenuate renal injury. A promising erythropoietin treatment has been proposed to reduce the need for red blood cell transfusion in HUS children. Use of antibiotics, antimotility agents, antithrombotic agents, narcotics and nonsteroidal anti-inflammatory drugs should be avoided during the acute phase. Immunization of cattle and humans, and new therapeutic protocols are under investigation, although none are ready for use in the near future. Thus, our weapons to cure HUS are still limited, and prevention is best done by preventing primary STEC infection. For this aim, more strict controls at all points of the food industry and a sustained educational campaign is mandatory, specially in Argentina, where there are over 400 new cases of HUS each year.

The hope that a better understanding of the pathogenesis and transmission routes of this disease will produce better therapies to prevent the acute mortality and long-term morbidity of HUS is the driving force for intensified research. Accumulating evidence from experimental therapies in animal models, and those from the few protocols assayed in humans suggest that the window for application of these new approaches is very small. In particular, the diagnosis of STEC should be made within 2 days after initiation of diarrhea, a challenge that cannot be achieved routinely, at least in Argentina. Therefore, improvement of community awareness of bloody diarrheas, new diagnostic tools and predictors for bad evolution are imperative to allow application of new therapeutic tools in those children at high risk of HUS development.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Key issues

- The typical or epidemic form of hemolytic uremic syndrome (HUS) is the major complication of gastrointestinal infections with seropathotypes of Shiga toxin (Stx)-producing *Escherichia coli* (STEC).
- HUS is a public-health problem in Argentina, since it is the second cause of chronic renal failure and accounts for 20% of renal transplantations in children and adolescents.
- Although Stx is the main virulence factor in STEC infections, additional factors from both the bacteria and the host would be necessary to lead to HUS in 10% of STEC infections. Among host factors, there is a great consensus about the importance of the inflammatory and thrombotic responses in the development of HUS.
- Volume expansion has been recently proposed during pre-HUS interval, as a new possibility to attenuate renal injury. On the contrary, the use of antimotility drugs or opioid narcotics has been discouraged as it is thought that these agents contribute to retention of Stx within the colon.
- Similarly, antibiotic treatment should be avoided, as beneficial effects regarding initiation of HUS cannot be deduced from recent studies.
- The early administration of erythropoietin has been recently proposed as a novel approach to reduce red blood cell transfusions in HUS children.
- In addition, there are experimental therapeutic strategies, including analogs of the globotriosylceramide receptor to be administered systemically and several anti-Stx antibodies that are being developed.
- Interventions in cattle are an interesting alternative to reduce the risk of human disease. They include modifications in animal feeding and management, use of probiotics and vaccination.
- Early diagnosis of STEC infection is essential for effective future therapy. Therefore, new diagnostic tools and predictors for bad evolution are imperative to allow application of new therapeutic tools in those children at high risk of HUS development.

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