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Pathogenesis of Shiga Toxin–Associated Hemolytic Uremic Syndrome

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ABSTRACT

The aim of this review is to examine recent advances in experimental and clinical research relevant to the pathogenesis of diarrhea-associated hemolytic uremic syndrome with special reference to histopathologic findings, virulence factors of Shiga toxin–producing *Escherichia coli*, the host response, and the prothrombotic state. Despite significant advances during the past decade, the exact mechanism by which Shiga toxin–producing *E. coli* leads to hemolytic uremic syndrome remains unclear. Factors such as Shiga toxin, lipopolysaccharide, the adhesins intimin and *E. coli*–secreted proteins A, B, and D, the 60-MD plasmid, and enterohemolysin likely contribute to the pathogenesis. Data on the inflammatory response of the host, including leukocytes and inflammatory mediators, are updated. The pathogenesis of the prothrombotic state leading to thrombocytopenia secondary to endothelial cell damage and platelet activation is also discussed. A hypothetical sequence of events from ingestion of the bacteria to the development of full-blown hemolytic uremic syndrome is proposed. **(*Pediatr Res* 50: 163–171, 2001)**

Abbreviations

HUS, hemolytic uremic syndrome
STEC, Shiga toxin–producing *E. coli* Stx, Shiga toxin
D^+ HUS, diarrhea-associated hemolytic uremic syndrome
D^- HUS, hemolytic uremic syndrome not associated with diarrhea
EHEC, enterohemorrhagic *E. coli* EPEC, enteropathogenic *E. coli*
Esps, *E. coli*–secreted proteins
LPS, lipopolysaccharide
Gb3, globotriaosylceramide
TNF-α, tumor necrosis factor-α

Nonimmune hemolytic anemia, thrombocytopenia, and acute renal failure are cardinal features of HUS (1). CNS manifestations may also be noted (2). HUS most frequently occurs among children younger than 5 years of age, after a diarrheal prodrome (D^+ HUS) typically characterized by bloody diarrhea, termed hemorrhagic colitis. This form of HUS may also occur in adults, more often among the elderly. In North America and Western Europe, Stx (also called verotoxin) -producing *E. coli*, most frequently involving the serotype O157:H7, is the leading cause of D^+ HUS (3–5). This review will address current knowledge about the pathogenesis of HUS caused by STEC.

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HISTOPATHOLOGY OF D^+ HUS

The histopathologic features observed in HUS have been termed thrombotic microangiopathy, a term that also encompasses other conditions such as HUS not associated with a diarrheal prodrome (D^- HUS) and thrombotic thrombocytopenic purpura (6). Endothelial cell damage is the hallmark of thrombotic microangiopathy found in D^+ HUS. Fibrin thrombi are predominantly formed within small vessels, followed by ischemic damage. Glomerular endothelial cell swelling, thrombotic occlusion of capillary lumens, tubular epithelial cell damage, mesangial expansion, and mesangiolysis have been observed (6). In the most severe cases, extensive cortical necrosis ensues. Inflammatory cell infiltrates within the kidneys are predominantly composed of neutrophils and macrophages (7, 8). Moreover, apoptosis of renal cortical glomerular and tubular cells is well documented (9, 10). Lesions of the gastrointestinal tract usually consist of small vessel angiopathy in the mucosa and submucosa of the small and large intestine with hemorrhage, necrosis, and sloughing of cells into the lumen (11). Thrombotic microangiopathy may also be noted in other organs such as the CNS (2). The histopathologic findings in D^- HUS and thrombotic thrombocytopenic purpura are

different from those in D^+ HUS. In D^- HUS, the lesions are arteriolar, with thrombi and intimal proliferation. In D^+ HUS the lesions are glomerular with occlusion of capillary lumens. There is mesangial expansion, and, in severe cases, cortical necrosis is noted (12, 13). Moreover, fibrin thrombi are characteristic of D^+ HUS, whereas platelets and von Willebrand factor constitute those seen in thrombotic thrombocytopenic purpura (14).

STEC VIRULENCE FACTORS

Strains of STEC that can cause hemorrhagic colitis and HUS have been termed EHEC. These include *E. coli* O157:H7 and many other serotypes. Food- and water-borne transmission are the most important means of infection (15–18), although person-to-person contamination has been recognized (19). Except for two case reports (20, 21), there is no evidence for bacteremia in human disease. It is therefore assumed that systemic spread of bacterial products may lead to damage of target organs. EHEC strains express Stxs, LPS, adhesins such as intimin and Esps A, B, and D, enterohemolysin, a serine protease EspP, and a heat-stable enterotoxin (22–27).

Shiga toxin. Stx has been implicated in the pathogenesis of HUS because cases have been associated with Stx-producing bacteria such as *E. coli* (22), *Shigella* (28), and *Citrobacter freundii* (29). On the other hand, EPEC, which possess many virulence factors found in EHEC, but not Stx, have never been associated with HUS.

Human isolates of STEC may express Stx1 or Stx2 encoded on a bacteriophage. The holotoxin includes one A subunit and five B subunits. The B subunit binds to glycosphingolipid receptors, predominantly Gb3 (30, 31). The A subunit has *N*-glycosidase activity, which leads to cell death by inhibition of protein synthesis at the level of 28S ribosomal RNA (32). Except for one amino acid substitution in the former, Stx1 from *E. coli* is virtually identical to the toxin produced by *Shigella dysenteriae* and approximately 60% homologous to Stx2. There are different pathways of translocation for Stx1 and Stx2 through the intestinal epithelium (33). Several observations suggest that Stx2 may be more virulent in human disease than Stx1. Clinical isolates of *E. coli* O157:H7 from patients with hemorrhagic colitis or HUS produced Stx2 more frequently than Stx1 (34–37). Moreover, an i.v. infusion of Stx2 in mice was found to be 400 times more potent than Stx1 (38). Similarly, human intestinal endothelial cells and renal glomerular endothelial cells are more sensitive to the cytotoxic effects of Stx2 than Stx1 (39, 40).

Stx-induced disease in animals. HUS preceded by hemorrhagic colitis has been described in several types of dogs as a naturally occurring disease (41). Greyhounds are known to develop a disease termed cutaneous and renal glomerular vasculopathy (Alabama rot), leading to an HUS-like condition (42, 43). STEC have been isolated in some cases of this canine disease (44). Edema disease in pigs causing vascular damage to the brain stem has been associated with Stx2e producing STEC (45). Swollen head syndrome is an acute respiratory disorder in poultry associated with STEC producing Stx2y (46). Although STEC cause disease in animals, no animal model has succeeded in reproducing all aspects of human disease after oral ingestion of the offending bacteria. Herein we will discuss several animal models that highlight aspects of human HUS. A more detailed description of animal models can be found in a current review (47).

In vivo *studies with Stx.* *In vivo* studies have indicated that STEC strains are pathogenic (48–54). The strains cause gastrointestinal, neurologic, or systemic symptoms and death in gnotobiotic piglets (50, 51, 55), rabbits (49), and mice (48, 52–54, 56). Inoculated animals developed histopathologic lesions such as inflammatory colitis (50), brain endothelial cell necrosis and neuronal impairment (52, 55), acute tubular necrosis, and mesangial expansion in the kidneys (48, 54, 56). In addition, mice inoculated intragastrically with *E. coli* O157:H7 had fragmented erythrocytes in the circulation (48), an aspect of disease that resembles human HUS. Comparison of toxin-positive with toxin-negative strains has shown that the former induces vascular colonic damage in a *Shigella* primate model (57) and glomerular mesangial changes, renal cortical apoptosis, and severe neurologic symptoms in an *E. coli* O157:H7 mouse model, changes not seen in animals infected with a toxin-negative strain (9, 48). The presence of Gb3 or galabiosyl ceramide receptors on cells has been found to determine the localization of tissue damage in rabbits, mice, and humans (38, 58, 59). Species-specific differences in the distribution of Gb3 receptors have been proposed to determine the localization of pathologic lesions in different animal models. The kidney and the CNS are richly endowed with Gb3, likely explaining the tissue tropism of Stx (60, 61).

In vivo experiments with purified Stx have reproduced aspects of HUS, with damage to the intestine, kidney, and CNS in rabbits and mice (62–64). Baboons infused with Stx1 develop renal failure, anemia, thrombocytopenia, and injury to intestinal epithelium, glomerular endothelial cells, and renal proximal tubular cells (65). In this experimental model, thrombi were noted in renal glomerular and peritubular capillaries, resembling the pathologic alterations seen in human HUS.

In vitro *studies with Stx.* Stx is cytotoxic for human endothelial cells (40, 66–71) and may also induce apoptosis (72). Prestimulation of endothelial cells with TNF-α, IL-1β, or sodium butyrate resulted in an increase in Gb3 receptors, rendering the cells more susceptible to the cytotoxic effect of Stx (70, 73–75). Stx may directly lead to endothelial cell activation with perturbed expression of endothelial-derived vasomediators (76). Both Stx1 and Stx2 directly activate bovine endothelial cells by increasing the production of preproendothelin mRNA transcript levels, without modifying that of nitric oxide (76). This effect was not observed with the receptor-binding B subunit, which lacks the *N*-glycosidase. In contrast, purified Stx was found to increase nitric oxide release from murine macrophages (77).

In addition to endothelial cells, Stx has been shown to have a cytotoxic effect on various other cells, including renal glomerular and tubular epithelial cells (78, 79). Cells lacking the toxin receptor were found to be resistant to the toxic effect (80). Stx can induce apoptosis in renal tubular epithelial cells (9, 81) as well as in Burkitt lymphoma cells (82), intestinal epithelial

cells (63, 83), pulmonary epithelium–derived cells (84), and Vero cells (85). Cell death was not only induced by the holotoxin but also by high doses of the B subunit, indicating that this effect may be independent of the effect on protein synthesis (82). Apoptosis was demonstrated in kidneys from patients with HUS and in mice inoculated with Stx-positive strains, but not Stx-negative strains (9). Taken together with the finding that Stx is capable of inducing apoptosis in renal tubular epithelial cells, these studies indicate that Stx-induced apoptosis may contribute to the renal injury during HUS. Apoptosis has been shown to be a causal mechanism in the pathogenesis of renal tubular injury and glomerular sclerosis (86–88).

Several studies have addressed the pathway by which Stx translocates from the intestinal lumen into the circulation. Stx1 translocation through intestinal epithelial cells occurs via a transcellular route, whereas that of Stx2 occurs through a paracellular pathway (33, 89). The toxin may also bind to intestinal endothelial cells (39) and, by damaging both epithelial and endothelial cells, induce hemorrhagic colitis and gain access to the bloodstream.

Lipopolysaccharide. Endotoxemia has been reported in patients with *Shigella* and other forms of non-STEC–induced HUS (28, 90) and has been implicated in the pathogenesis of HUS. Endotoxemia has not been found in patients with STEC-associated HUS. In contrast to *Shigella*, STEC are noninvasive bacteria. Indirect evidence of LPS expression *in vivo* has been shown by circulating antibodies against LPS in patients (91). Furthermore, compared with children with uncomplicated O157:H7 enteritis, those with HUS present an increased acute phase response to LPS, as measured by circulating LPS-binding protein (92). These findings do not, however, indicate lipid A (endotoxin) expression.

Animal models have also indicated that LPS may play a role in the pathogenesis of HUS. Rabbits injected with LPS exhibited renal cortical necrosis, resembling the renal lesions found in HUS (93, 94). The procoagulant events in HUS may resemble the generalized Schwartzman reaction, observed after i.v. infusion of LPS in rabbits (93, 94). However, the lack of disseminated intravascular coagulation and endotoxic shock in HUS indicates that these correspond to different pathophysiologic processes.

Pretreatment of rabbits and mice with Stx enhanced the lethal effects of LPS (64, 95). LPS hyporesponsiveness was associated with a biphasic course of disease in mice inoculated with wild-type *E. coli* O157:H7 (48) and a longer time to death in mice injected with Stx2 (96). These studies indicate a synergistic effect of Stx and LPS *in vivo*, as has also been shown *in vitro* using endothelial cells (68). Other reports observed that pretreatment with LPS could either increase or decrease Stx2-induced lethality, depending on the dose and timing of injection and that the effects could be modulated by TNF-α or IL-1β (97). A recent publication (98) showed that antibodies to LPS block adherence of STEC to human intestinal epithelial cells *in vitro*, although the biologic significance of this finding has not been evaluated *in vivo*.

Adhesins. Using *in vitro* cultures of human intestine, it has been shown that the initial binding of *E. coli* O157:H7 occurs in the follicle-associated epithelium of Peyer's patches within the small bowel (99). Colonization of the colon may occur thereafter (99). Adhesins are responsible for pathogen binding to intestinal epithelial cells and, as such, may be a critical step in the pathogenesis of hemorrhagic colitis (100). As with EPEC, EHEC strains exhibit attachment in a localized manner, termed attaching and effacing adherence (101). This form of intimate adherence involves the effacement of intestinal microvilli and accumulation of polymerized actin (102) and other cytoskeletal components at the site of bacterial attachment. It is mediated by intimin, an outer membrane protein (103, 104), encoded by the *eae* gene as well as by Esps A, B, and D, which mediate epithelial cell signal transduction (26, 105). All factors necessary for the formation of these lesions, including a translocated intimin receptor (tir, also termed EspE) (106, 107) are encoded in the locus of enterocyte effacement on the bacterial chromosome (26). A detailed review of these adherence mechanisms has been published recently (108). The expression of the type III secretion apparatus required for the development of attaching and effacing lesions is regulated by quorum sensing (109), a mechanism by which bacteria regulate their own population and that of other bacteria in their immediate surroundings. This may indicate that colonization of the intestine by *E. coli* O157:H7, ingested at low doses, may be induced and modulated by nonpathogenic *E. coli* in the human intestine.

60-MD plasmid, enterohemolysins, and heat-stable enterotoxin. *E. coli* O157 H7 and most other EHEC strains possess a large 60-MD plasmid (25, 110). Human isolates were more often positive for *eae* and EHEC plasmid sequences than animal isolates, suggesting that these factors together with other bacterial factors may be required for full virulence of the strain (111). This plasmid was found to encode for an enterohemolysin and some biologic activity of adherence, presumably mediated by fimbriae (112). Nonpiliated mutants could, however, also adhere, and the importance of the plasmid for adherence is not clear (113). Recently, an extracellular serine protease EspP, encoded by the 60-MD plasmid, was found capable of cleaving pepsin A and human coagulation factor V (24). Although the presence of the plasmid has been associated with clinical disease, the precise pathogenic role, if any, of the enterohemolysins and the large plasmid have not been elucidated. All strains of *E. coli* O157:H7 and many other STEC possess the *astA* gene encoding for the heat-stable enterotoxin EAST-1 (27). Its role also remains unknown.

HOST RESPONSE

Extensive tissue injury occurs during hemorrhagic colitis and HUS, thereby generating an inflammatory response through leukocyte activation and cytokine production.

Polymorphonuclear and mononuclear cells. Several observations suggest that neutrophils and macrophages may play a key role in the pathogenesis of disease. HUS is frequently associated with circulating leukocytosis (114–125). Furthermore, this has been shown to be an independent risk factor for developing HUS (114, 116, 117). Increased circulating neutrophil (119, 120) and macrophage (120) counts are associated with the severity of renal failure during HUS. An increased

number of neutrophils and macrophages have been found within the glomeruli of children with HUS, compared with controls (7, 8). Increased elastase levels are found in the blood of HUS patients (121, 124, 125), indicating neutrophil activation. Circulating polymorphonuclear leukocytes are activated (121–123). Although the underlying mechanisms leading to circulating leukocytosis remain unclear, increased circulating levels of granulocyte colony-stimulating factor (126) and IL-8 (124) have been reported in children with HUS. Moreover, IL-8 levels correlated with the white blood cell count (124). Neutrophils from patients with HUS have a higher capacity to adhere to cultured human endothelium and to induce endothelial injury by degradation of endothelial fibronectin (122). The latter may be blocked by anti-CD18 MAb (122).

Mice injected with Stx developed elevated neutrophil counts. These neutrophils exhibited enhanced adhesive and cytotoxic properties, and pretreatment of mice with LPS potentiated this effect (127). *In vitro*, Stx1 increases the number of leukocytes adhering to endothelial cells, an effect that may be enhanced by TNF-α (128). Stx1 binds to a non-Gb3 receptor on human polymorphonuclear cells (129). In the presence of human glomerular microvascular endothelial cells *in vitro*, Stx1 will transfer from polymorphonuclear cells to the endothelial cells. It has thus been suggested that neutrophils may transport Stx from the gut to the kidney vasculature (129). In support of this hypothesis, Stx did not induce apoptosis of neutrophils, suggesting that neutrophils are resistant to the cytotoxic effects of Stx (130).

Monocytes may play a central role in the pathogenesis of HUS, as depletion of hepatic or splenic macrophages, using clodronate, reduced Stx2 cytotoxicity in mice (131). Stx1 binds to human monocytes via a different Gb3 subtype than on endothelial cells and leads to the secretion of TNF-α, IL-1β, IL-6, and IL-8 via an LPS-independent pathway (132). Other investigators also found that Stx1 activated TNF-α production and gene transcription in a human monocytic cell line (133, 134). These studies show that Stx, in addition to being cytotoxic to human cells, is capable of triggering cells to produce and release cytokines.

Circulating inflammatory mediators and their role in STEC-induced disease. Various circulating inflammatory mediators, including ILs, chemokines, soluble adhesion molecules, growth factors, cytokine receptors, and acute phase response proteins, are abnormally increased in children with D^+ HUS (7, 92, 124, 126, 135–148). Although these studies do not prove that the elevated inflammatory mediators have a role in the pathogenesis of disease, they indicate a marked host inflammatory response. In addition, elevated circulating levels of inflammatory mediators may be related to decreased renal excretion. Supporting this view, it has been reported that the half-life of IL-10 is increased in anephric mice (149). Patients with HUS have elevated circulating levels of pro- (IL-6, IL-8) and antiinflammatory (IL-10, IL-1 receptor antagonist) cytokines in comparison to patients with uncomplicated *E. coli* O157:H7 enteritis (135). A correlation to the severity of renal dysfunction has been made. Concentrations of IL-6, IL-8, IL-10, and the IL-1 receptor antagonist were higher during the acute phase of HUS in patients with oliguria and those requiring dialysis as well as in patients with lower GFR 1 year after development of HUS (136). In other studies, IL-6 concentrations correlated with anuria and extrarenal manifestations (136–138) during the acute phase of disease. Sequential samples of serum IL-6 correlated with disease activity as measured by serum creatinine, Hb, and platelet counts, the highest levels being found during the first days after onset of anuria, normalizing at recovery (138). Evidence from clinical studies and models of renal disease indicate that IL-6 may be involved in mesangial proliferation (150, 151) and may thus be elevated in response to glomerular damage.

Certain mediators may be involved in chemotaxis. IL-8, a known neutrophil chemoattractant, was significantly elevated in the plasma of children with HUS and correlated to neutrophil counts (124). The highest concentrations were detected in the blood of patients who died during the acute phase of disease (124). Stx may induce IL-8 secretion from intestinal epithelial cells (152, 153) and thus prime the inflammatory response in the intestine, leading to increased chemotaxis and further activation of inflammatory cells. Increased serum levels of granulocyte colony-stimulating factor (126, 143) and monocyte chemoattractant protein-1 (7) have also been found.

TNF-α is a key proinflammatory cytokine that mediates inflammation and microvascular coagulation (150). Increased concentrations of TNF-α (138, 140, 141, 143, 145) and soluble TNF receptors (137) have been observed in HUS. There is evidence for a role of TNF-α in the pathogenesis of HUS. *In vivo* and *in vitro* studies have shown that Stx and TNF-α act in concert to induce renal cell injury. In mice Stx was found to induce TNF synthesis in kidneys and to increase the sensitivity to the toxic effects of TNF-α (64). Inhibition of TNF-α by nafamostat mesylate reduced the renal and CNS histologic changes in mice infected with *E. coli* O157:H7 (154). Stx infusion in baboons led to increased urinary secretion of TNF-α and IL-6 (65). *In vitro* experiments have shown that Stx1 may stimulate the production of proinflammatory cytokines within the proximal tubule, including TNF-α, IL-1, and IL-6 (155). Preincubation of human tubular epithelial cells with TNF-α increased Stx-induced apoptosis (9). Stx and TNF-α or IL-1β act in synergy to induce a cytotoxic effect on endothelial cells (67, 71, 73). Pretreatment of human saphenous vein endothelial cells with TNF-α before stimulation with Stx1 induced secretion of von Willebrand factor (70), which may be involved in the process of intravascular platelet aggregation. In addition, TNF-α increased adherence of leukocytes to Stx-stimulated endothelial cells and up-regulated adhesion proteins on the endothelial surface membrane (128), a process that could contribute to leukocyte-mediated endothelial cell damage in HUS.

In addition to elevated inflammatory mediators in the circulation, TNF-α, IL-6, IL-8, monocyte chemotactic protein-1, basic fibroblast growth factor, and platelet activating factor have been found to be elevated in the urine of patients with D^+ HUS (7, 138, 140, 141, 156, 157). The kinetics of the serum cytokine response differed from that in urine, and there was no correlation between serum and urine levels in the same individual, suggesting that these cytokines are produced locally within the kidney, and not filtered from the bloodstream (138,

140). Most cytokines are peptides of low molecular weight, which are filtered from serum to urine (158). The concentration of filtered substances may be higher in urine than blood owing to reabsorption of water in the tubular epithelium. The uremic kidney with both glomerular and tubular damage would not be capable of such reabsorption. Thus we assume that the cytokines in the circulation and those in the urine are derived from different sources.

Endothelin is a vasoconstrictive peptide found to be excreted in a variety of renal diseases (159). Abnormally high levels of endothelin have been reported in the serum and urine of patients with HUS (144, 147, 160), reflecting the degree of renal injury, but possibly contributing to it by vasoactive potency.

Certain lymphokines such as IL-2, IL-4, and IL-13 were undetectable during *E. coli* O157:H7 enteritis or HUS, and low concentrations of interferon-γ were comparable among children with hemorrhagic colitis and HUS and control subjects, suggesting that T lymphocytes are not activated (139). Decreased levels of soluble L-selectin were observed in children with HUS, perhaps reflecting the appearance of immature neutrophils in the circulation (135). Levels of soluble E-selectin, P-selectin, and vascular cell adhesion molecule-1 were analyzed in several studies (135, 142, 146) and were not consistently found to be altered in comparison to controls. Finally, children with uncomplicated *E. coli* O157:H7 enteritis were found to have higher serum concentrations of transforming growth factor β1 than those who develop HUS, possibly related to intestinal repair mechanisms (139) and gastrointestinal spillover.

PROTHROMBOTIC STATE

Thrombocytopenia is a cardinal feature of HUS. Platelets are consumed in microthrombi, and circulating platelets are degranulated (161) with an impaired aggregating ability and decreased intracellular levels of β-thromboglobulin (162). Platelet-derived microvesicles are increased, indicating their state of activation (163). HUS plasma induces aggregation of normal platelets (164), and increased platelet-derived factors such as platelet factor 4, β-thromboglobulin, and P-selectin have been found (165, 166). Studies have attempted to identify a direct interaction between platelets and Stx. The toxin binds to the Gb3 receptor and to a glycolipid termed band 0.03 on platelets (167). Culture filtrates from STEC were able to induce platelet aggregation (168). Although purified Stx does not induce platelet aggregation in an aggregometer (169–171), a recent study has shown that Stx and its B subunit bind to platelets leading to direct activation. In the presence of human umbilical vein endothelial cells, pretreated with TNF-α, Stx and the B subunit induce the formation of aggregates on these cells (172). Thus, platelet consumption during HUS may be related to a direct effect of Stx on platelets or may be related to Stx-induced endothelial cell injury, exposing the subendothelium. The latter may release prothrombotic substances such as von Willebrand factor and fibrinogen, thereby leading to platelet aggregation, which will in turn obscure the vessel lumen in target organs, leading to ischemic damage. Markers of endothelial cell activation, such as tissue plasminogen activator and plasminogen activator inhibitor 1, are increased, indicating a prothrombotic and hypofibrinolytic state (173).

There is no consumption of coagulation factors in HUS. Prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, has been found to be deficient in certain cases of HUS (174, 175). Deficiency may be caused by low production secondary to damaged endothelium, or by high consumption, and may promote platelet aggregation. Thromboxane is a potent vasoconstrictor that is significantly elevated during acute HUS (176).

Hemolytic anemia. Fragmentation of erythrocytes is common during the acute hemolysis seen in HUS. This may be caused by mechanical breakdown along the damaged endothelium (177). However, oxidative damage to red blood cells has also been suggested (178). Animal models have reproduced this aspect of HUS using either wild-type bacteria (48) or purified Stx (65). Using the former, endothelial cell damage could not be demonstrated, and it was therefore suggested that red blood cell fragmentation may occur independently of endothelial injury.

A HYPOTHETICAL DESCRIPTION OF THE SEQUENCE OF EVENTS LEADING TO HUS

A schematic overview of the proposed mechanisms underlying the pathogenesis of STEC-associated HUS is shown in Figure 1. STEC are ingested and establish along the intestine. The exact localization of intestinal colonization has not been defined, although recent studies using *in vitro* organ cultures

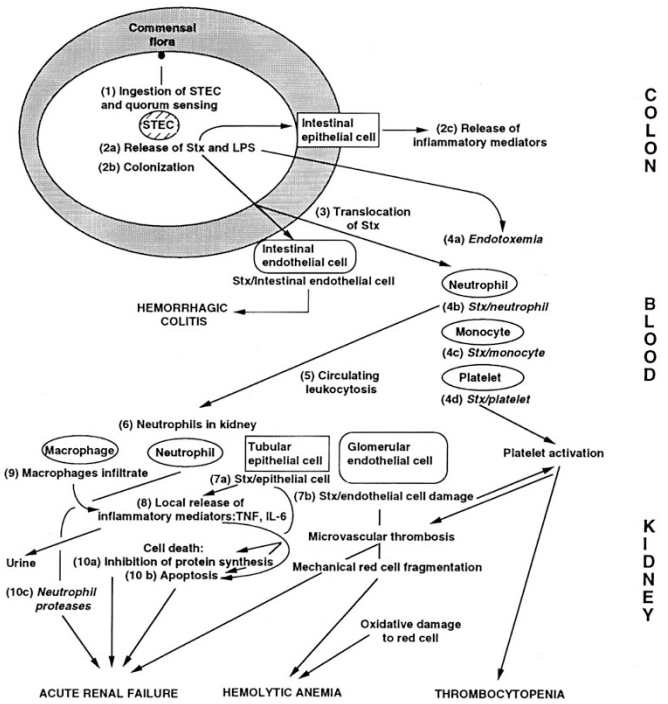

Figure 1. Overview of a hypothetical sequence of events from ingestion of STEC to the development of HUS. Numbers-1–10 give the order of events. Events which may occur simultaneously are mentioned with a small letter, *e.g.* 2a, 2b. Hypotheses for which data are either lacking or pending are shown in italics. The proposed pathophysiologic mechanisms are discussed in the text.

suggest that EHEC initially attach to Peyer's patches, after which colonization of the colon may occur (99). Quorum sensing regulates the expression of adhesins so that even small amounts of ingested bacteria are capable of adhering (109). Adhesion occurs by the translocation of Esps from the bacterium into the host cell, leading to signal transduction and activation. Transfer of the translocated intimin receptor into the host cell enables intimate binding of intimin to the intestinal epithelium (108). Furthermore, bacteria secrete Stx, LPS, and possibly other virulence factors into the intestinal lumen and intestinal epithelial and endothelial cells (33, 39, 89). This eventually leads to colitis with bloody diarrhea owing to cell death and intestinal vascular injury (39). Intestinal cells are activated to secrete cytokines, such as IL-8, locally and into the circulation (124, 152). The kinetics of the toxin-induced inflammatory response and the role of individual cytokines have yet to be determined. Stx, and possibly LPS and other virulence factors, gains access to the circulation. The mechanism by which Stx circulates and reaches its target organs has not been clarified, although binding to monocytes, polymorphonuclear cells, or platelets has been proposed (129, 132, 167, 172). On reaching the microvasculature of the kidneys (179), brain, and other Gb3-endowed organs, the toxin binds to its receptor and induces stimulatory effects, leading to the local release of cytokines such as TNF-α, IL-1, and IL-6 (155), and cytotoxic effects (67–69, 78), followed by cell death. The released cytokines lead to further influx of inflammatory cells that may also be triggered to release cytokines (132–134), and in synergy with LPS and Stx, this will increase the cytotoxic damage (67–69, 73). Stx is thus capable of stimulating cells to release cytokines as well as inducing cell death by inhibition of protein synthesis (32) or by apoptosis (9, 81). Monocytes, polymorphonuclear cells, and platelets have, however, been found to be resistant to the cytotoxic effects of Stx (129, 132, 172). Although the cellular events that regulate Stx-triggered cell activation *versus* death have, as yet, not been elucidated, it has been suggested that cytotoxicity may be subsequent to binding to the Gb3 receptor and that binding via non-Gb3 receptors or Gb3 receptors with a different fatty acid composition will not necessarily mediate cell death (129, 132). Platelet consumption occurs because of direct activation by Stx and intravascular aggregation (172) or secondary to endothelial cell injury (177). Activated platelets in areas of high shear and damaged endothelium may bind to the subendothelium. Unbound platelet aggregates are removed by the reticuloendothelial cell system. Platelet consumption and removal may thus lead to thrombocytopenia. Glomerular thrombi and damage to tubular epithelial cells (6) will lower glomerular filtration and cause renal failure. Red blood cell fragmentation secondary to mechanical injury in the vasculature (177) or to oxidative damage (178) leads to hemolysis. It is notable that not every individual ingesting EHEC will exhibit symptoms. Host factors that have been proposed to contribute to HUS are young or old age (19, 180) and the P1 blood group (181, 182), although other, as yet unknown host factors likely also play a role.

CONCLUSIONS

This review presents advances in our current understanding of the pathogenesis of STEC-induced disease. Although the precise sequence of events leading from ingestion of bacteria to the development of HUS is still unknown, and a completely valid animal model is lacking, new data have significantly improved our knowledge and the entire *E. coli* O157:H7 genome was recently sequenced, enabling future identification of genetic sequences related to pathogenesis (183). Small quantities of bacteria may colonize the intestine by a bacterial cross-talk mechanism termed quorum sensing (109). The mechanisms of intestinal adhesion have been characterized (108). Stx is uniquely associated with bacteria that cause HUS, and together with host factors and inflammatory mediators, contributes to the target organ injury. The toxin has been found to be both stimulatory and cytotoxic and may circulate bound to polymorphonuclear cells from which it will transfer to endothelial cells (129). Stx has a cytotoxic effect on endothelial cells (66, 67) and has most recently been shown to activate platelets (172). These advances will hopefully enable the development of toxin binding, neutralizing, and removal therapies in the future.

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