Enterohemorrhagic
Escherichia coli Pathogenesis
and the Host Response

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ABSTRACT Enterohemorrhagic Escherichia coli (EHEC) is a highly pathogenic bacterial strain capable of causing watery or bloody diarrhea, the latter termed hemorrhagic colitis, and hemolytic-uremic syndrome (HUS). HUS is defined as the simultaneous development of non-immune hemolytic anemia, thrombocytopenia, and acute renal failure. The mechanism by which EHEC bacteria colonize and cause severe colitis, followed by renal failure with activated blood cells, as well as neurological symptoms, involves the interaction of bacterial virulence factors and specific pathogen-associated molecular patterns with host cells as well as the host response. The innate immune host response comprises the release of antimicrobial peptides as well as cytokines and chemokines in addition to activation and/or injury to leukocytes, platelets, and erythrocytes and activation of the complement system. Some of the bacterial interactions with the host may be protective in nature, but, when excessive, contribute to extensive tissue injury, inflammation, and thrombosis, effects that may worsen the clinical outcome of EHEC infection. This article describes aspects of the host response occurring during EHEC infection and their effects on specific organs.

INTRODUCTION

Bacterial exotoxins may cause damage to host cells by defined mechanisms. Depending on the presence of the globothriaosylceramide (Gb3) receptor, Shiga toxin may bind to cells and induce the ribotoxic stress response and apoptosis (1, 2). The toxin can also induce a pro-inflammatory response in cells, an effect that may be dissociated from ribosome inactivation and can even occur in cells lacking protein synthesis machinery. Bacterial lipopolysaccharide (LPS) induces a host response by binding to Toll-like receptor 4 (TLR4) and activating specific intracellular pathways. The activation of pro-inflammatory pathways, if excessive, promotes damage to the host. This article addresses enterohemorrhagic Escherichia coli (EHEC) pathogenesis and the host response, examining the innate and adaptive immune responses to the bacteria and virulence factors and how they affect the process of colonization, transfer and transport of virulence factors in the circulation, activation of thrombosis and inflammation, and specific end-organ damage to the kidney and the brain.

EHEC COLONIZATION AND THE HOST RESPONSE IN THE INTESTINE

During intestinal colonization EHEC strains encounter chemical, mechanical, and biological barriers (3). Chemical encounters include saliva containing mucins and enzymes, acid stress in the stomach, bile secretion in the small intestine, and antimicrobial peptides throughout the intestine. The mechanical barrier consists of the mucus layer, and the biological barrier includes the intestinal microflora. These encounters, and the innate and acquired immune response to the pathogen, attempt to eliminate the bacteria. EHEC strains must overcome...
these barriers to colonize. After ingestion, strains survive stomach acidity by expressing acid-resistant systems (4–6). Interestingly, the response to acid stress is not only a survival strategy but was also found to activate certain EHEC properties associated with enhanced motility and cell adhesion, but not to affect the expression of Shiga toxin (Stx) (4, 6). From the stomach the bacteria pass to the small intestine where contact with bile may further promote migration (6).

Initial binding is assumed to occur at the follicle-associated epithelium of Peyer’s patches and villi of the terminal ileum (7, 8). Bacteria may be taken up by intestinal M cells and transferred to underlying macrophages where they can survive and produce Stx (9). In the small and large intestine the bacteria come in contact with short-chain fatty acids, acetate, propionate, and butyrate, secreted by the intestinal flora as fermentation products of dietary carbohydrates (10). Low concentrations of butyrate were shown to upregulate EHEC virulence genes involved in motility and formation of attaching and effacing (A/E) lesions (11). This effect of butyrate was abrogated by deletion of the bacterial hpa gene encoding the leucine-responsive regulatory protein. High concentrations of short-chain fatty acids trigger expression of the bacterial iha gene conferring colonic adherence (12). Furthermore, butyrate treatment of colon cells in vitro resulted in enhanced expression of the Stx receptor and thus increased susceptibility to the toxin (13).

Short-chain fatty acids also affect the expression of antimicrobial peptides, cathelicidin and defensins (14), thus creating a hostile environment for bacterial colonization, as shown by using the A/E-forming pathogen Citrobacter rodentium (15). In a mouse model inoculated with EHEC, cathelicidin protected wild-type mice from EHEC colonization, infection, and subsequent renal injury in comparison to knockout mice (3). The effect occurred at the intestinal level, as cathelicidin-deficient mice were not protected from the effects of injected Stx. In addition to enhanced bacterial survival, cathelicidin-deficient mice had a thinner colonic mucus layer and thus a defective intestinal mechanical barrier. Although antimicrobial peptides protect the host from bacterial colonization, colonic pathogens may actually downregulate antimicrobial peptides, as demonstrated in shigellosis (16), to the advantage of the bacteria.

The interaction with the intestinal commensal microflora also activates communication between bacteria known as quorum sensing and between bacteria and host hormones. The interaction of bacteria with host catecholamines promotes virulence by activating bacterial motility (flagellar synthesis), formation of A/E lesions, and increased expression of Stx (17, 18). Presumably an increase in catecholamines in the circulation and the local intestinal environment could occur during hemorrhagic colitis.

The bacteria release Stx into the intestinal lumen and onto enterocytes. Toxin was demonstrated inside intestinal cells from a patient with EHEC infection, indicating that it can be taken up by these cells (19). These cells may express the Gb3 receptor to which the toxin binds (13, 20). The toxin may, however, undergo endocytosis by macrophages (19). Furthermore, the toxin binds to intestinal Paneth cells (21). Alternatively, excess permeability of the mucosal barrier may allow Stx transport from the lumen in a paracellular fashion (22) although this remains a speculation. Thus the precise manner by which Stx binds to intestinal cells and is internalized or transported to the endothelium in the in vivo setting, is as yet, unclear. Stx leads to apoptosis of epithelial cells in vitro in human (23–25) and mouse intestinal cells (26). Apoptosis was demonstrated in the human intestine affected by EHEC infection, in a rabbit in vivo model (27), and in the murine EHEC model. The major virulence factor associated with this effect was determined to be Stx (28). The apoptosis-inducing effect initiates an inflammatory response and leukocyte influx of primarily phagocytes. Neutrophil-inducing effect initiates an inflammatory response and leukocyte influx of primarily phagocytes. Neutrophil migration toward the intestinal lumen occurs simultaneously and enhances Stx translocation from the lumen via enterocytes in vitro (29, 30). High leukocyte counts were demonstrated in feces of patients with E. coli O157:H7 infection (31), suggesting that a similar process may occur in vivo.

The intestinal inflammatory response is a paramount feature of host resistance to infection. The initial interaction between the intestinal mucosa and bacterial virulence factors may promote bacterial clearance by inducing an appropriate degree of inflammation. Invasive enteropathogenic strains induce an excessive release of chemokines from intestinal cell lines in comparison to noninvasive strains, including E. coli O157:H7 (32). All the same, in vitro studies have demonstrated release of interleukin-8 (IL-8) by T84 intestinal cells stimulated with EHEC, thus promoting inflammatory influx. Stx alone can also induce secretion of IL-8 and other C-X-C chemokines in the gut (33–35). Stx-induced IL-8 expression was associated with induction of mitogen-activated protein (MAP) kinase pathways Jun N-terminal protein kinase and stress-activated protein kinase and p38 in intestinal epithelial cells (23). Thus the apparently disparate proinflammatory and apoptotic pathways may converge via induction of host stress-activated
MAP kinases. Stx induces the ribotoxic stress response, thereby inhibiting protein synthesis, but may, via eIF4E phosphorylation, promote translation of inflammatory mediators, so that both processes occur simultaneously \(36\). Stx also induced the expression of tumor necrosis factor alpha (TNF-\(\alpha\)) and IL-6 from murine peritoneal macrophages \(37\). In addition to Stx, long-polar fimbriae expressed by EHEC were recently shown to induce a proinflammatory response in T84 cells in a study showing that the NF-kB pathway was activated \(38\).

EHEC could, however, suppress the intestinal epithelial cytokine response to Stx \(39\). Likewise, EHEC, and Stx in particular, were shown to inhibit gamma interferon-mediated epithelial cell activation \(40\); both these effects could mitigate the host response and thus potentially promote bacterial colonization.

The importance of LPS in EHEC infection was demonstrated in a murine model using C3H/HeJ LPS-hypo-responsive mice in comparison to wild-type C3H/HeN mice. C3H/HeN mice developed earlier and simultaneous systemic and neurologic symptoms whereas C3H/HeJ mice exhibited a biphasic course of disease, first developing systemic symptoms and later severe neurologic symptoms \(41\). The discrepancy between LPS-responders and non-responders was assumed to be related to the initial intestinal inflammatory response. LPS may induce a mucosal immune response during the initial phase of disease, which could facilitate bacterial clearance. A reduced initial response, due to lack of response to LPS, would promote more severe disease as clearance of bacteria from the intestine would be delayed, enabling bacterial proliferation and extended toxin release intestinally and systemically, thus explaining the biphasic prolonged course of disease in C3H/HeJ mice.

Pathogen-associated molecular patterns, or PAMPs, are specific pathogen-associated molecules recognized by cell receptors, such as TLRs, transmembrane receptors within the innate immune system. Stimulation of TLRs triggers a downstream cellular signal that results in the production and release of cytokines and chemokines. TLRs recruit intracellular adaptor proteins. MyD88 is an adaptor molecule common for all TLRs except for TLR3. LPS binds to the MD2-TLR4 receptor complex and initiates a signal cascade via MyD88-dependent or MyD88-independent pathways \(42\). TLR signaling depends on four adaptor proteins—MyD88, TIRAP (also known as MAL), TRIF (also known as TICAM1), and TRAM (also known as TICAM2)—that recruit downstream signaling components \(43\).

The importance of TLR4, TRIF, and MyD88 for the pathogenesis of EHEC infection was demonstrated in wild-type and knockout mice infected with \textit{E. coli} O157:H7 (both Stx2-producing and non-producing) \(44\). Only Stx2-producing mice developed symptoms, and the most severe symptoms and pathology were demonstrated in MyD88-deficient mice. These mice also had the highest bacterial burden, suggesting that the immune response at the intestinal mucosa was essential for bacterial elimination. Even TLR4-knockout mice exhibited more severe disease than wild-type mice did. In contrast, an in vitro study showed that Stx uses TLR4 on intestinal cells for cellular uptake and transport \(45\); thus lack of TLR4 should have been protective in vivo, which was not the case.

THE ACQUIRED IMMUNE RESPONSE TO EHEC INFECTION

An acquired immune response develops after EHEC infection. In developing countries, enteropathogenic \textit{E. coli} (EPEC) is a similar pathogen capable of causing diarrhea. In similarity to EHEC, it has a type-3 secretion system and can thus form A/E lesions on the intestinal epithelium, but EPEC does not produce Stx. Antibodies against antigens common to both EPEC and EHEC strains, such as \textit{E. coli}-secreted proteins A and B as well as intimin, have been found in human serum, saliva, colostrum, and breast-milk \(46\–52\).

EHEC-infected patients were also found to have serum and saliva antibodies against the infecting strain’s LPS \(53\,54\) although the antibody levels decreased over time \(55\). Anti-LPS antibodies were also detected in umbilical cord sera of uninfected women in areas of EPEC endemicity \(56\). Patients may likewise develop serum antibodies against Stx2 and Stx1 \(57\), and a lesser antibody response to Stx and LPS was even detected in asymptomatic household contacts \(58\).

To what degree anti-Stx and anti-LPS antibodies exert a protective effect against infection is unclear. However, antibodies against common EPEC and EHEC antigens may have a protective effect and thus explain the low prevalence of EHEC infections in areas where EPEC is endemic \(59\). Colostrum from Brazilian mothers in areas of EPEC endemicity was shown to inhibit adherence of EHEC strains to HEp-2 cells \(52\). The protective effect of cross-immunity between EPEC and EHEC was demonstrated in a mouse model in which mice were first inoculated with EPEC, followed by inoculation with EHEC \(60\). Mice prechallenged with EPEC developed antibodies against common antigens and were protected from symptoms and pathology (intestinal and renal) caused by EHEC infection.
**SHIGA TOXIN AND LPS INTERACTIONS WITH BLOOD CELLS**

**Neutrophils**

Neutrophils are a prominent component of the acute inflammatory response, and levels of circulating neutrophils rise during an infectious process. In HUS, neutrophil counts are usually elevated at presentation (61), and the degree of neutrophilia at the onset of HUS is predictive of outcome (62). Neutrophils in HUS are activated and degranulated, thus releasing proteases (63, 64), as demonstrated by the presence of high levels of neutrophil elastase in patient sera (63, 66) and a higher capacity to adhere to cultured human endothelial cells and degrade fibronectin (67). Degranulation and activation of neutrophils correlated with poor prognosis (68, 69). Moreover, patient neutrophils were also delayed in their apoptotic program with an increased life span (63, 70).

Augmented levels of circulating apoptotic and necrotic leukocytes were found in patients with *E. coli* O104:H4-associated HUS (71). Plasma levels of leukocyte-derived microparticles were elevated as was binding of platelet microparticles to leukocytes. Inhibition of complement had only a moderate impact on microparticle secretion of proinflammatory cytokines and chemokines such as CD11b, CD64, CD62L, and CX3CR, possibly due to monocyte infiltration of tissue lesions (81, 82).

Patients with HUS were found to have Stx2 on monocytes and platelet-monocyte aggregates (78). Monocytes express small amounts of the Gb3 receptor that is slightly different from the Gb3 lipofoms present on endothelial cells (83). The number of Gb3 receptors on monocytes can be increased by LPS binding, thus leading to activation of the cells and enhanced binding of Stx (83). Stx is not cytotoxic for monocytes but triggers a variety of proinflammatory events, including in vitro synthesis and secretion of proinflammatory cytokines and chemokines such as IL-6, IL-8, TNF-α, and IL-1β (83).

Monocytes may indirectly contribute to the thrombotic process occurring during HUS. Stimulation of macrophage-like cells of the monocytic cell line THP-1 with Stx2 induced the release of macrophage-derived chemokine, RANTES, and IL-8, an effect that was further enhanced in the presence of LPS, leading to platelet activation and aggregation (84). Incubation of monocytes with Stx1 or Stx2 induced expression of tissue factor on their surfaces (78, 85), which was further enhanced upon coincubation with LPS and when monocytes were in complex with platelets (78), and thus presumably involved in the prothrombotic process occurring during HUS.

Monocyte-derived microparticles expressing tissue factor were detected in patients with HUS (78). Stx2 induced the release of tissue factor-expressing microparticles from monocytes in vitro. Monocyte-derived microparticles can deliver and transfer tissue factor to platelets (86) and neutrophils (87) and thus induce a prothrombotic surface. Taken together, Stx can bind to and activate monocytes and induce the formation of platelet-monocyte aggregates and the release of tissue factor-expressing microparticles with prothrombotic properties. However, the transfer of Stx from monocytes to endothelial cells probably does not occur in vivo (88), as both cells express receptors with similar affinities (83).

**Monocytes**

Monocytes are a critical effector component in the innate immune response by presenting antigens and producing cytokines, thus regulating innate and adaptive immune responses. In HUS, monocytes are differentiated toward an inflammatory phenotype with an increased population of cells with reduced CD14 and enhanced CD16 membrane expression (81). In addition, monocytes from patients with HUS exhibited reduced circulatory expression of function-related proteins such as CD11b, CD64, CD62L, and CX3CR, possibly due to monocyte infiltration of tissue lesions (81, 82).
Platelets

Low platelet counts and formation of renal thrombi are characteristic of HUS, suggesting that platelet activation is involved in the pathogenesis. Thrombocytopenia is assumed to be related to consumption of platelets in microthrombi and may be caused by the direct effects of Stx on platelets or by Stx-induced endothelial cell damage leading to secondary platelet activation and the formation of microthrombi \( (89, 90) \). During the acute phase of HUS platelets are degranulated \( (91) \), with reduced intracellular levels of β-thromboglobulin and impaired aggregating response \( (92) \). Platelet-derived microparticles are increased, indicating platelet activation \( (78, 93) \), and plasma from patients with HUS induced aggregation of normal platelets \( (94) \). Mice inoculated with \( E. coli \) O157:H7 mimicked the human disease and developed thrombocytopenia, which was also demonstrated in mice injected with Stx2 and LPS \( (44, 95) \).

Platelets bind Stx through Gb3 and an alternative glycosphingolipid receptor, termed band 0.03 \( (96, 97) \). Gb3 expression on resting platelets is very low \( (97) \), and the distribution in humans is quite heterogeneous \( (96) \). Stx is assumed to bind primarily to activated platelets \( (78, 97) \), and Stx circulates bound to platelets, in addition to leukocytes, during HUS \( (98) \). Upon binding to platelets, Stx is rapidly internalized, leading to further activation, aggregation, structural changes enhancing the surface area, and increased fibrinogen-binding capacity \( (99) \).

In addition to their role in thrombosis and hemostasis, platelets are involved in inflammation and can influence both innate and adaptive immunity \( (100) \). Activated platelets interact with both neutrophils \( (101) \) and monocytes \( (102) \) and release chemokines such as platelet factor-4, macrophage inflammatory protein (MIP), RANTES, IL-8, β-thromboglobulin, and monocyte chemoattractant protein (MCP) from their α-granules, which potentiate the inflammatory process \( (103) \).

Both human and murine platelets express TLR4 and other TLR receptors \( (98, 104, 105) \). Platelet TLRs mediate LPS-induced thrombocytopenia and TNF-α production by leukocytes \( (105-109) \). Recent evidence suggests that platelets may bridge the innate and adaptive immune system by expressing immunostimulatory proteins such as CD40L and thereby stimulate CD8+ T-cell induction and adaptive immunity \( (110) \). Resting platelets must be primed (with LPS or other activators) before interaction with Stx \( (78, 111) \). LPS binds to platelets via a receptor complex of TLR4 and CD62 and activates them as shown by the expression of CD40L, activated GPIIb/IIIa receptor, and fibrinogen binding \( (98) \). GPIIb/IIIa and CD40L both play a central role in thrombotic diseases. In addition, CD40L can interact with CD40 on endothelial cells, triggering an inflammatory response leading to local or systemic release of MCP-1, VCAM, and ICAM \( (112-114) \).

LPS INTERACTION WITH BLOOD CELLS

Recognition of LPS by innate immune cells is vital for host defense against gram-negative bacteria. LPS, the major component of the outer membrane of gram-negative bacteria, circulates bound to platelets during acute HUS \( (98) \). Platelets from mice inoculated intraperitoneally with LPS showed surface-bound LPS and exhibited increased CD40L expression, suggesting that LPS activates platelets in the circulation in the murine model \( (98) \). Patients develop an antibody response to strain-specific LPS \( (115) \). The concentration of LPS-binding protein, which binds LPS in plasma and transfers it to cell surfaces, is increased in the plasma of patients with HUS compared to those with uncomplicated EHEC diarrhea, suggesting that the acute-phase response to LPS is associated with disease severity \( (116) \).

Neutrophils and monocytes express TLR4 and respond to LPS stimulation by releasing proinflammatory cytokines \( (117) \). At the onset of HUS, neutrophils exhibited higher levels of TLR4 mRNA and TLR4 protein expression \( (118) \). TLR4 expression was correlated to increased circulating TNF-α levels. No differences were noted in TLR4 receptor expression on monocytes at the onset of HUS \( (118) \), indicating different regulation of TLR4 expression on neutrophils and monocytes.

TISSUE FACTOR

During the acute phase of HUS, patients have high plasma levels of tissue factor and tissue factor pathway inhibitor \( (119) \) and circulating platelet-leukocyte aggregates expressing tissue factor and tissue factor-bearing platelet microparticles \( (78) \). In vitro studies showed that Stx, in cooperation with LPS, induced aggregate formation between platelets and leukocytes, leading to release of platelet-derived microparticles with surface-bound tissue factor \( (78) \).

Tissue factor-positive microparticles may contribute to thrombosis. Tissue factor is a transmembrane glycoprotein receptor \( (120) \) for coagulation factor VII \( (121) \). By acting as a cofactor for factor VII, tissue factor...
promotes proteolysis and activation of factor VIIa, followed by formation of the prothrombinase complex (122) and conversion of prothrombin into thrombin, resulting in thrombus formation and further platelet activation. Expression of tissue factor on platelets is debated. Platelets contain tissue factor mRNA (123), which can be spliced into mature mRNA upon platelet activation, leading to minimal protein expression and procoagulant activity (124, 125). However, platelets seem to acquire most of their tissue factor through interaction with tissue factor-bearing microparticles from monocytes (126, 127).

THE THROMBOTIC PROCESS IN HUS

The prothrombotic condition in HUS has been primarily ascribed to damage of the microvascular endothelium. When vascular injury occurs, platelets are recruited to the damaged site in a multistep process that involves the interaction of specific platelet cell-surface receptors with subendothelial matrix proteins such as von Willebrand factor (VWF), collagen, and fibronectin. The first event in this process is binding of platelet glycoprotein 1bα receptor (GP1bα) to the A1 domain of VWF after which a conformational change in the platelet integrin receptor GPIIb/IIIa occurs, allowing binding to both VWF and fibrinogen, inducing aggregation. After initial tethering steps, platelets become activated and firmly adhere to the vessel wall and form a clot. Adherent and activated platelets release potent platelet agonists such as thrombin, ADP, and thromboxane A2 from their intracellular granules, promoting further platelet activation and aggregation and resulting in rapid growth of the thrombus.

In HUS, platelets are activated (128) and degranulated (91, 92), and platelet-derived factors such as β-thrombogobulin and platelet factor-4 are elevated (129) as is VWF, which may be secreted from both platelets and endothelial cells (130, 131). VWF mediates platelet adhesion to activated endothelial cells in response to Stx (132). In addition, Stx delayed the cleavage of VWF-platelet strings by the metalloprotease ADAMTS13 on activated endothelial cells (133), thus potentiating thrombus growth in the presence of larger VWF multimers. Functional blockade of receptors or adhesive proteins, such as GPIIb/IIIa, P-selectin, or VWF, was associated with a marked reduction of thrombi on endothelial cells (132). Both Stx and LPS activate platelets, especially under high shear stress, and costimulation with both factors simultaneously induced an additive effect on the formation of platelet-leukocyte aggregates expressing tissue factor (78, 97–99). In addition, platelets may be activated indirectly by additional factors such as chemokines and cytokines released by Stx-stimulated monocytes (84) or endothelial cells (134). Thus, platelet activation and thrombus formation may be caused directly by Stx and/or LPS or by the release of cytokines, indicating that platelet activation and inflammation are correlated events.

In parallel with the recruitment of platelets, the blood coagulation cascade is activated at the site of vessel injury (135). In HUS, there is no consumption of coagulation factors, but elevated levels of prothrombin fragment 1 + 2 (136–138), tissue plasminogen activator, tissue plasminogen activator inhibitor-1 (139), and D-dimers have been found, even before HUS develops, indicating enhanced thrombotic capacity and impaired fibrinolysis.

THE SYSTEMIC AND RENAL HOST RESPONSE

The precise mechanism by which Stx and other EHEC virulence factors, such as LPS, reach the kidney is, as yet, unknown. Although Stx and O157LPS have been shown to circulate bound to blood cells, as described above, the manner by which they transfer to resident target organ cells in the kidney, brain, or other organs has not been elucidated in the in vivo setting. However, the toxin was detected in glomeruli and tubuli of pediatric and geriatric patients with HUS (140, 141), indicating that it reaches the kidney. The extensive renal injury associated with renal cell apoptosis occurring during HUS (142) activates a variety of host responses, including the influx of leukocytes and the release of cytokines, both of which could enhance the tissue damage.

Leukocytosis was associated with the development of HUS (143, 144) and with a poor outcome (145), as described above. Renal influx of neutrophils was associated with increased mortality (61, 146). The functionality of neutrophils correlated to patient renal dysfunction (68). Mice that were coinfected with Stx2 and LPS developed neutrophilia and monocytosis with markers of leukocyte activation (95). Neutrophil and macrophage accumulation was demonstrated in the murine kidneys (147, 148). Likewise, kidneys from rabbits injected with Stx2 showed PMN infiltrates correlating with levels of IL-8 (149). In vitro studies showed that stimulation of glomerular endothelial cells with Stx2 enhanced leukocyte adherence and migration under perfusion, effects mediated by MCP-1, IL-8, and fractalkine (CX3CL1) secreted from the cells (150, 151) and enhanced by TNF-α (152). Furthermore, Stx borne
on leukocytes migrated through endothelial cells and induced their release of IL-8 and MCP-1 (153).

In addition to leukocytes, platelets have also been demonstrated in the glomerular microthrombi characteristic of human thrombotic microangiopathy (reviewed in reference 154), in the glomeruli of primates treated with Stx (155) and of mice injected with Stx2 and LPS (95). Low platelet counts during HUS are correlated to poor recovery of renal function (62, 156). Stx and LPS induce human microvascular endothelial cells to secrete factors that activate platelets (134). Although platelets are activated and secrete microparticles during HUS, and the role of platelets in the renal thrombotic events is evident, their contribution to the proinflammatory events occurring in the kidney has not been thoroughly investigated. For example, degranulation of platelet alpha granules upon activation will release microbicidal proteins, CC-chemokines, and CXC-chemokines (103, 154). The binding of specific ligands and release of potent platelet components could theoretically promote the inflammatory state in the kidney.

Proinflammatory and prothrombotic responses have been documented in HUS. Inflammatory and prothrombotic mediators, including cytokines, chemokines, soluble adhesion molecules, growth factors, cytokine receptors, tissue factor, and acute-phase response proteins, are elevated in patients with EHEC-associated infection and HUS (116, 119, 138, 157–174) and could be correlated, in certain studies, to the progression of renal damage (reviewed in reference 89). Increased levels of chemoattractants, such as IL-8 (69), granulocyte colony-stimulating factor (159), and MCP-1 (157), could explain leukocyte influx. Stx and TNF-α act in synergy to cause cytotoxic effects on human endothelial cells (175–177). Elevated TNF-α may enhance the expression of the Stx receptor Gb3 on endothelial cells (178). Enhanced Gb3 expression was also demonstrated on cultured endothelial cells stimulated with LPS and IL-1 (175, 177, 179, 180), thus sensitizing the cells to Stx.

Animal models have further addressed the importance of inflammatory and chemotactic pathways for the pathogenesis of HUS. Gnotobiotic mice inoculated with E. coli O157:H7 exhibited TNF-α, IL-1, and IL-6 in the kidney (181). Certain mice were also treated with TNF-α, which led to enhanced kidney damage. TNF inhibition, with the protease inhibitor Nafamostat mesilate, reduced target-organ damage. Stx induced TNF synthesis in the mouse kidney while increasing renal sensitivity to the toxic effects of TNF (182). Interestingly, TNF had a protective effect when administered to mice before Stx1 (183) but exacerbated disease when given after Stx1. These results are in contrast to a previous study using neutralizing anti-TNF-α antibody before administration of Stx1, as well as TNF-α knockout mice, which could not demonstrate a role for TNF-α in Stx-induced toxicity (184). In vitro results indicated that Stx induced increased secretion of TNF-α from human renal proximal tubule cells (185) and that Stx2-induced TNF-α expression was diminished by blocking the p38 pathway (186).

HUS patients exhibited high levels of TNF-α in the circulation (163, 166, 168, 170) and in the urine, which did not correlate to levels in the blood, indicating local synthesis in the urinary tract (164). TNF-α is a pro-inflammatory cytokine mediating a cytotoxic effect on tumor cells, inflammation, and microvascular coagulation. The high urinary levels occurring during HUS (164) may contribute to inflammation, thrombosis, and end-organ damage.

A recent study using baboons treated with Stx exhibited high renal mRNA levels of IL-8, MCP-1, and MIP-1α in contrast to a modest effect on TNF-α. These baboons had elevated urinary IL-6, IL-8, MCP-1, and VEGF (155). Similarly, a previous study in baboons showed that Stx infusion led to urinary secretion of TNF-α and IL-6 (187). Stimulation of proximal tubular cells with Stx increased expression of IL-6 mRNA and protein (185). A similar effect was noted in stimulated glomerular endothelial cells, particularly when cells were costimulated with LPS (188). Patients with HUS exhibit very high IL-6 levels in serum and urine (164). Elevated glomerular and tubular levels of IL-6 may promote renal injury. In addition to its role in immune regulation and inflammation, IL-6 may respond to glomerular injury by promoting mesangial proliferation (189).

The chemokine receptor CCR1 was shown to be involved in neutrophil and monocyte infiltrates in the kidney and in host survival after Stx2 treatment of CCR1-deficient mice (190). A slower increase in plasma TNF-α and IL-6 was also noted in the CCR1−/− mice compared to wild-type mice. Similarly, injection of Stx2 and LPS in a mouse model markedly increased renal expression of the chemoattractants CXCL1 and CXCL2, affecting neutrophil influx (147). In the same mouse model, macrophage influx was also demonstrated and associated with expression of monocyte chemoattractants: MCP-1/CCL2, MIP-1/CCL3, and RANTES (CCL5) (148). Taken together, the studies described provide evidence for chemokine- and cytokine-mediated inflammation that underscores the importance of the inflammatory response in the development of HUS.
The CXCR4/CXCR7/stromal cell-derived factor 1 (SDF-1) pathway, involved in renal homeostasis, was activated by Stx2 (174), thus enhancing endothelial activation and renal damage. The results were confirmed by using human microvascular endothelial cells in vitro and showed that low concentrations of Stx, which minimally affect protein synthesis, activated the CXCR4/CXCR7/SDF-1 pathway (134, 174). The importance of this finding was established in patients showing elevated plasma levels of SDF-1 and thus suggested an effect on the glomerular vasculature.

Tissue factor is a potent prothrombotic mediator and was found to be elevated in the plasma of HUS patients (119, 173), on circulating platelet-derived microparticles (78), and demonstrated in the kidney (191). Stx induced tissue factor activity in glomerular endothelial cells and proximal tubular cells, an effect enhanced in the presence of TNF-α (192, 193).

THE HOST RESPONSE IN THE BRAIN

The central nervous system (CNS) is also a target organ during HUS, and as many as 48 to 66% of patients developed severe neurological manifestations during the E. coli O104:H4 epidemic in Germany in the spring of 2011 (194, 195). Shiga toxin may bind to the Gb3 receptor on neurons and endothelial cells in the human CNS (196). The induction of apoptosis (197, 198) and the inflammatory response may promote CNS injury. In a study conducted on pediatric patients with HUS and encephalopathy, versus patients with HUS without CNS symptoms or with EHEC-associated colitis without HUS, serum inflammatory mediators IL-6, soluble TNF receptor 1, and tissue inhibitor of metalloproteinase-1 were correlated to the presence of encephalopathy (199). Increased protein in the cerebrospinal fluid (200) also indicates an enhanced inflammatory state.

EHEC strains were used in animal models to reproduce neurological affection (41, 201) showing endothelial and neuronal damage, and Stx was detected in the brain cortex and spinal cord (201). The blood-brain barrier is generally affected by damage to cerebral capillary endothelial cells, perivascular pericytes, and possibly even astrocytes. Astrogliaosis is triggered by multiple inflammatory mediators (202). In EHEC-infected mice astrocytes were activated in the medulla oblongata and spinal cord (203). EHEC-infected mice exhibited TNF-α in the brain, and intraperitoneal treatment with TNF-α given before and after EHEC inoculation worsened the neurological symptoms. The TNF inhibitor, Nafamostat mesilate, modified these responses and decreased cytokines in the brain, suggesting a role for TNF-α in the neurological manifestations (181). Furthermore, intravenous injection of Stx2 in rabbits induced brain edema that could be reversed by anti-inflammatory steroid treatment (204).

In vitro experiments using human brain endothelial cells have shown that TNF-α and IL-1β enhanced Stx toxicity (205). TNF increases the Gb3 receptor on human brain endothelium (206). The TNF effect was mitigated by inhibition of p38 MAP kinase (207). Similarly, TNF-α amplified the inflammatory response to Stx1 and LPS-treated rat astrocytes, enhancing PMN chemotaxis and cytotoxicity (208). Inflammatory mediators released from the stimulated astrocytes affected endothelial permeability and increased binding of PMNs and platelets to the endothelial cells (209). Thus in vivo and in vitro evidence points to a considerable inflammatory response in the CNS affecting the endothelium and astrocytes as well as the blood-brain barrier.

THE EFFECTS OF COMPLEMENT ACTIVATION

Complement activation via the alternative pathway occurs during EHEC-associated HUS. This has been documented by hypocomplementemia (low C3) and elevated plasma levels of degradation products such as complement factors Bb, C3a, and soluble C5b-9 during the acute phase of disease; this did not correlate with the severity of renal injury or occurrence of later complications and decreased upon recovery (210–213). However, circulating C3a and soluble C5b-9 may activate platelets (214, 215). One patient was also shown to have C3 deposits on circulating platelet-monocyte aggregates (213). Patient platelet- and monocyte-derived microparticles were shown to be coated with C3 and C9 deposits during the acute phase of HUS but not after recovery (213). These findings suggest that the extensive endothelial and blood cell activation occurring during HUS will lead to secondary complement activation. A direct effect of Stx and/or O157/LPS was demonstrated when these were incubated in vitro with whole blood, leading to the formation of leukocyte-platelet aggregates and the release of platelet- and monocyte-derived microparticles, both with C3 and C9 deposits (213). Complement could also be activated by dysfunctional inhibition. Stx was shown to activate the alternative pathway in vitro and to bind to cell-binding domains of the major soluble complement inhibitor, factor H, thus compromising the inhibitory effect and promoting complement activation (216). These studies...
were performed with very high concentrations of Stx2 and may thus not reflect the in vivo situation. Also, as the toxin does not circulate in free form, but is rather cell-bound in the circulation, the interaction with factor H may not occur in vivo (213). Factor H prevents complement activation via the alternative pathway on cell surfaces. Factor H binding to proximal tubular cells was impaired when cells were exposed to protein overload, resembling the situation during renal injury (217). Thus complement activation may also occur as a nonspecific consequence of tubular protein overload.

One study addressed activation of the mannann-binding lectin (MBL) pathway in EHEC-induced infection and HUS. MBL deficiency may predispose to infection, but no correlation was found between EHEC infection and MBL deficiency (218). The main function of the complement system is to dispose of foreign cells, such as bacterial pathogens, by opsonization and cytolysis (219). Complement is active in the colon (220), and its primary effect there is to promote bacterial clearance. Thus a certain degree of complement activation is protective at the mucosal surface. However, prolonged and amplified activation will promote platelet activation and endothelial damage in HUS (219, 221).

**SUMMARY**

The host responds to EHEC infections and, more specifically, to Shiga toxin and EHEC-derived LPS by activation of a large variety of cells, including blood cells, intestinal and renal epithelial cells, renal and cerebral endothelial cells, and astrocytes. Multiple chemokines and cytokines, as well as stress hormones and antimicrobial peptides, are secreted. They contribute to the inflammation and interact with bacteria or virulence factors in an attempt to clear the infection. The inflammatory responses may, however, have adverse effects on the host, thus worsening the outcome. In certain instances the bacterial virulence factors may hijack host responses to their own advantage. The release of tissue factor and thrombin promotes thrombosis, and the complement system is activated with secondary effects on the endothelium, tubular cells, and platelets. The inflammation and thrombosis arising explain some of the features of HUS. It is thus essential to understand not only the cellular signaling pathways used by EHEC virulence factors but also the pattern of inflammatory and thrombotic responses arising from the infection.

Treatments aimed at reducing injury to host organs should primarily attempt to neutralize Shiga toxin. However, as patients may manifest profound organ damage, treatments aimed at reducing the bacterial load, the inflammatory and thrombotic response, and the cell injury may be beneficial if instituted early in the course of infection. The specific pathways leading to host cell damage should therefore be determined, so that future treatments can effectively abrogate the inflammatory and thrombotic complications of this infection.

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**REFERENCES**


