Published in final edited form as:
Curr Top Microbiol Immunol. 2012 ; 357: 105–136. doi:10.1007/82_2011_172.

Shiga Toxin Pathogenesis: Kidney Complications and Renal Failure

Tom G. Obrig and
Department of Microbiology and Immunology, University of Maryland, School of Medicine, 685 West Baltimore Street, HSF-I Suite 380, Baltimore, MD 21201, USA

Diana Karpman
Department of Pediatrics, Clinical Sciences Lund, Lund University, Lund, Sweden

Tom G. Obrig: tobrig@som.umaryland.edu; Diana Karpman: Diana.Karpman@med.lu.se

Abstract

The kidneys are the major organs affected in diarrhea-associated hemolytic uremic syndrome (D^+HUS). The pathophysiology of renal disease in D^+HUS is largely the result of the interaction between bacterial virulence factors such as Shiga toxin and lipopolysaccharide and host cells in the kidney and in the blood circulation. This chapter describes in detail the current knowledge of how these bacterial toxins may lead to kidney disease and renal failure. The toxin receptors expressed by specific blood and resident renal cell types are also discussed as are the actions of the toxins on these cells.

This review provides a detailed view of the current knowledge of Shiga toxin (Stx) actions and host responses that comprise the disease known as diarrhea-associated hemolytic uremic syndrome, D^+HUS. Stx2 is the predominant form of Stx produced by clinical isolates of STEC and is considered to be the form of Stx that causes D^+HUS in humans. Expression of the Stx receptor, glycosphingolipid Gb3, or a close analog, is required for Stx interaction with eukaryotic cells. Stx targets specific cell types in the kidney and blood circulation causing the cells to either become activated, elicit proinflammatory mediators, or apoptose. The primary target of Stx is thought to be endothelial cells, however, additional Stx-sensitive cell types exist in the kidney that are required for normal filtration of the blood (endothelial, podocyte) and fluid balance (tubules). Damage to these cells helps explain how Stx causes proteinuria, dehydration, and renal failure. Stx-sensitive platelets in the blood appear to be central to the renal vascular coagulation and thrombosis of D^+HUS. Recent evidence indicates that bacterial lipopolysaccharide (LPS) is required for platelet activation in D^+HUS prior to their interaction with Stx. The role of specific cytokines and chemokines in the kidneys and blood circulation remains to be determined for D^+HUS. Limited data support the concept that transport of Stx from the colon to the kidneys may involve neutrophils. Although successful delineation of the pathophysiology of D^+HUS in humans will continue to depend on animal models of D^+HUS, none of the animal models currently available for D^+HUS completely recapitulates the disease in humans.

1 Shiga Toxin Interaction With Blood Cells in the Circulation

Stx-producing *Escherichia coli* (STEC) are non-invasive. Stx is released in the intestine and after passing through the intestinal mucosal barrier the toxin circulates in the bloodstream

Correspondence to: Tom G. Obrig, tobrig@som.umaryland.edu.

before reaching its target organs. The main target organ is the kidney. How the toxin circulates and whether it is free in the circulation or cell-bound has been addressed in numerous studies. In patients with hemolytic uremic syndrome minimal amounts of Stx were found in the serum (Brigotti et al. 2011) but in mice infected with *E. coli* O157:H7 Stx was detected in serum 2–5 days after inoculation (Kurioka et al. 1998). The presence of Stx in the circulation in mice but not in humans may reflect the longer time span that elapses from infection to clinical presentation in patients. In addition to free toxin, which may no longer be present in the circulation when HUS develops, studies have shown that toxin may bind to blood cells and thus reach its target organs. Stx is not cytotoxic for neutrophils, monocytes, and certain B lymphocytes (Cohen et al. 1990; van Setten et al. 1996; Liu et al. 1999a; Brigotti et al. 2008). Platelets and red blood cells (RBCs) are presumed to be less sensitive to the cytotoxic effects of Stx as they lack nuclei. Thus binding of the toxin to these blood cells will not destroy the cells.

1.1 Platelets

Low platelet counts are a hallmark of HUS and lower levels are associated with worse renal prognosis (Robson et al. 1988). Mice inoculated with STEC developed thrombocytopenia which was also demonstrated in mice injected with Stx2 and LPS (Keepers et al. 2006; Calderon Toledo et al. 2008). Thrombocytopenia may be induced by multiple mechanisms such as consumption of platelets in microthrombi on the surface of damaged endothelium (Zoja et al. 2010), activation by circulating chemokines or other circulatory platelet aggregating factors and by a direct interaction between STEC virulence factors and platelets leading to their aggregation and deposition on endothelial cells (Karpman et al. 2001; Ghosh et al. 2004; Guessous et al. 2005b; Ståhl et al. 2006; Zoja et al. 2010).

Stx circulates in vivo bound to platelets during HUS (Ståhl et al. 2006). The toxin binds to activated platelets (Ghosh et al. 2004) via the globotriaosylceramide (Gb3) receptor and an alternative glycosphingolipid receptor termed band 0.03 (Cooling et al. 1998; Ghosh et al. 2004). Stx undergoes endocytosis and exerts an activating effect on platelets which readily aggregate on endothelial cells (Karpman et al. 2001). There is, to date, no evidence that Stx is transferred from platelets to cells in target organs.

1.2 Monocytes

Stx1 binds to monocytes via a Gb_3 receptor that differs somewhat from the receptor on endothelial cells. Binding was enhanced in the presence of LPS and did not inhibit protein synthesis (van Setten et al. 1996). On the contrary, Stx induced the synthesis and release of cytokines IL-1β, IL-6, IL-8, and TNF-α from human monocytes in vitro (van Setten et al. 1996). Similar results were obtained using a monocytic cell line THP-1 which, upon stimulation with Stx2, released IL-8, macrophage-derived chemokine (MDC), and regulated upon activation, normal T-cell expressed and secreted (RANTES) capable of activating platelets (Guessous et al. 2005a). A role in the prothrombotic manifestations occurring during HUS was suggested by the finding that Stx1 and Stx2 could induce the expression of tissue factor on monocytes (Murata et al. 2006; Ståhl et al. 2009). Tissue factor expression was increased when Stx2 was co-incubated with LPS and particularly when monocytes were in complex with platelets (Ståhl et al. 2009). Patients with STEC-associated HUS were found to have Stx2 on platelet-monocyte complexes. Furthermore, Stx2 induced the release of monocytic microparticles bearing tissue factor and tissue factor expressing-microparticles were detected in patients (Ståhl et al. 2009). Tissue factor-bearing microparticles may fuse with platelets and thus induce a pro-thrombotic process (Del Conde et al. 2005).

Although Stx can bind to and activate monocytes, and induce the formation of platelet-monocyte complexes with prothrombotic properties, monocytes have not been shown to

assist in the circulatory transfer of toxin to target cells (Geelen et al. 2007a, b). All the same, biopsies from patients with HUS have demonstrated that monocytes infiltrate the kidneys most probably recruited by increased levels of monocyte chemoattractant protein-1 (van Setten et al. 1998).

1.3 Neutrophils

Stx was detected on the surface of neutrophils from HUS patients (Te Loo et al. 2001; Tazzari et al. 2004; Ståhl et al. 2009; Brigotti et al. 2011). In addition, Stx2 was detected on the surface of neutrophil-platelet complexes in whole blood from HUS patients (Ståhl et al. 2009). Binding of Stx to neutrophils has been debated (Geelen et al. 2007a), especially as the Stx receptor on these cells has still not been identified (van Setten et al. 1996; Geelen et al. 2007a; Arfilli et al. 2010). Toxin bound preferentially to mature PMNs (Brigotti et al. 2008). In vitro studies have shown that Stx could transfer from the surface of neutrophils to human endothelial cells suggesting that neutrophils could serve as a carrier for the toxin until the target organ was reached (Te Loo et al. 2000; Brigotti et al. 2010).

Neutrophil counts are high during HUS and higher levels, as well as elevated IL-8 levels, correlated with poor prognosis (Fitzpatrick et al. 1992; Robson et al. 1992; Fernandez et al. 2007). High PMN levels could be partially attributed to decreased spontaneous neutrophil apoptosis during HUS (Fernandez et al. 2007). Stx2 may promote neutrophilia in mice by triggering the release of cells of myeloid lineage from the bone marrow and by accelerating proliferation of PMN progenitors (Fernandez et al. 2006). Stx2 was also shown to impair neutrophil migration in mice (Fernandez et al. 2006). Neutrophils are activated during STEC infection. Circulating neutrophils degranulate thus releasing proteases and reactive oxygen species (Fernandez et al. 2005). In vitro studies have shown that Stx2 activates neutrophils, particularly those in complex with platelets (Ståhl et al. 2009). Furthermore, leukocytes adhere to the endothelium in the presence of Stx, a process promoted by endothelial expression of fractalkine, MCP-1, IL-8, and heparan sulfate proteoglycans (Zoja et al. 2002; Geelen et al. 2008; Zanchi et al. 2008).

In addition to Stx, *E. coli* O157 may also secrete StcE, a metalloprotease that cleaves mucin demonstrated to increase neutrophil oxidative burst and cell adhesion, thus impairing neutrophil migration (Szabady et al. 2009). Taken together, the interactions between Stx or StcE and neutrophils could explain neutrophil activation, degranulation, and impaired migration leading to increased tissue destruction at sites of neutrophil infiltration. Furthermore, the possibility that neutrophils bear Stx on their membrane during HUS would enable transfer of the toxin to other target cells more prone to a cytotoxic reaction.

1.4 Red Blood Cells

Fragmented RBCs occur during HUS in which non-immune hemolytic anemia occurs. It is assumed that RBCs fragment mechanically while passing through partially occluded capillaries. One study also reported that RBC membranes were stiffened by oxidative damage (Turi et al. 1994). Although the exact mechanism by which hemolysis occurs is, as yet unclear, the RBC fragmentation can be reproduced in animal models inoculated with *E. coli* O157:H7 (Karpman et al. 1997) or injected with Stx (Taylor et al. 1999).

Hemolysin is one of the virulence factors expressed by *E. coli* O157:H7 encoded in a large plasmid (pO157) (Schmidt et al. 1994). The hemolysin is a pore-forming cytolysin. *E. coli* 0157:H7 hemolysin has been shown to lyse human RBCs in vitro (Bauer and Welch 1996), but this phenomenon most probably does not occur in vivo as bacteremia does not occur during STEC infection and STEC hemolysin is bound to the bacterial cell membrane (Schmidt et al. 1994; Bauer and Welch 1996).

Studies have investigated whether the expression of different P blood group antigens on RBCs may influence the risk of developing HUS. The P blood group includes three different antigens: P, P1, and Pk, giving rise to five different phenotypes P1, P2, P1k, P2k, and p. The Pk antigen is the precursor of the P antigen as well as the Gb3 receptor for Stx. Thus the Gb3 receptor for Stx is present on all RBCs that possess the Pk antigen (all but the rare p phenotype) (Bitzan et al. 1994; Spitalnik and Spitalnik 1995). Studies have addressed whether expression of the P1 blood group could be protective reducing the risk of developing HUS based on the assumption that P1 expression on RBCs would bind Stx and thereby reduce the amount of circulating toxin. Thus individuals with RBCs negative for P1 or with lower amounts of Gb3 would be at higher risk of developing HUS (Taylor et al. 1990; Newburg et al. 1993). These findings however, could not be confirmed by others (Jelacic et al. 2002).

2 Gb3 Expression in the Kidney

Specific and high-affinity binding of Stx to its receptor is required for toxin action in the disease process. The receptor for Stx1 and Stx2 is the glycosphingolipid Gb3 that is expressed in kidneys and some other tissues of humans and animals (McCluer et al. 1981; Cohen et al. 1987; Lindberg et al. 1987; Lingwood et al. 1987; Boyd and Lingwood 1989; Obrig et al. 1993; Lingwood 1996; Hughes et al. 2002; Garcia et al. 2006). Gb3 is synthesized within golgi of cells, then transported to and inserted into the plasma membrane outer leaflet where the trisaccharide faces outward for specific recognition by the B-subunits of Stx (Lingwood 1993). The importance of Gb3 for Stx action was revealed by the total absence of Stx effects in mice lacking Gb3 and, in vitro, by concomitant reconstitution of Gb3 and Stx-sensitivity in cells lacking Gb3 (Waddell et al. 1990; Okuda et al. 2006). Recent advances in glycolipid biology indicate that Gb3 is localized to lipid rafts rich in cholesterol (Hakomori 2000; Falguieres et al. 2001; Falguieres et al. 2006; Muthing et al. 2009). Binding of Stx to Gb3 localized to lipid rafts is important for action of Stx in eukaryotic cells (Hoey et al. 2003; Smith et al. 2006a; Khan et al. 2009; Lingwood et al. 2010a). Human kidney contains a series of Gb3 subspecies which differ in their ceramide hydrocarbon chain length (C16–C24) and degree of hydroxylation (Shayman and Radin 1991; Muthing et al. 2009). Recent data support the concept that not all Gb3 in the plasma membrane is bioavailable (Johannes and Romer 2010; Lingwood et al. 2010a, b; Mahfoud et al. 2010; Betz et al. 2011).

2.1 Glomerular Gb3

It is generally accepted that the renal endothelium is a primary target of Stx in STEC-associated hemolytic uremic syndrome, also known as D^+HUS, in which the D^+ stand for diarrhea (Zoja et al. 2001; Karch et al. 2005; Ahn et al. 2009). Changes in the renal endothelium typically include swelling and detachment from the basement membrane (Habib 1992). Human glomerular microvascular endothelial cells have been shown to be sensitive to sub-nanomolar Stx, in vitro (Obrig et al. 1993; van Setten et al. 1997a, b). This is in contrast to human large vessel umbilical vein endothelial cells that are relatively refractory to Stx action (Obrig et al. 1988, 1993; Tesh et al. 1991). This differential sensitivity to Stx was demonstrated to be due to a 50-fold higher expression of Gb3 by the glomerular endothelial cells (Obrig et al. 1993). As described below in more detail, human podocytes and mesangial cells are also sensitive to Stx (Simon et al. 1998; Psotka et al. 2009). Human podocytes and mesangial cells express Gb3 (Robinson et al. 1995; Psotka et al. 2009).

Animal models for D^+HUS should preferably reflect the Gb3 expression pattern of human kidneys. However, an analysis of Gb3 location in kidneys of such animals remains incomplete although the mouse, rat, pig, rabbit, dog, ferret, and baboon have been utilized as

models of D^+HUS (Taylor et al. 1999; Rutjes et al. 2002; Melton-Celsa and O'Brien 2003; Caprioli et al. 2010). None of these models completely recapitulates the D^+HUS renal disease of humans. Presently, the baboon model of D^+HUS appears best in this regard following intravenous administration of purified Stx2 (Taylor et al. 1999; Siegler et al. 2003; Stearns-Kurosawa et al. 2010). However, details of location and quantification of Gb3 in baboon kidney have yet to be reported.

2.2 Extraglomerular Gb3

There is a need for a thorough assessment of Gb3 expression along the different segments of the nephron in human and animal kidneys. Most of the available data for Gb3 expression in tubules are derived from isolated propagated cell types of human and animal kidneys. Numerous studies have demonstrated the significant Stx-sensitivity of tubule cells, but little information is presented about Gb3, per se, in these cells in culture (Hughes et al. 1998a; Kodama et al. 1999; Liu et al. 1999b; Williams et al. 1999; Kaneko et al. 2001; Nestoridi et al. 2005a; Paixao-Cavalcante et al. 2009). Analysis of intact renal tissues from human and animal sources provided evidence that Gb3 is expressed by renal tubules (Lingwood 1994; Rutjes et al. 2002; Ergonul et al. 2003a; Winter et al. 2004; Silberstein et al. 2008). Direct binding of Stx to frozen sections of murine kidney revealed Gb3 in proximal, distal, and collecting duct cells (Tesh et al. 1993; Rutjes et al. 2002). In human kidney sections, Stx binding was localized to distal and collecting duct tubule epithelium (Lingwood 1994). A more detailed examination of human and murine renal tissue performed with co-localization of Gb3 with renal tubule cell-type specific markers indicated that Gb3 is expressed by proximal and collecting duct tubules in both human and murine kidney (Psotka et al. 2009) (Obata and Obrig, unpublished data). Interstitial microvascular endothelial cells also express Gb3. The effects of Stx on renal tubule cells are described in more detail in the following section.

3 Stx Interaction With Cell Types of the Kidney

Much information is now available regarding Stx interaction with resident cells of the kidney (Karpman et al. 2010; Obrig 2010). The fact that Stx interacts with many cell types of the kidney makes difficult the assignment of their relative importance for development of renal disease in D^+HUS.

3.1 Glomerular Cells

As described above, human glomerular podocyte, endothelial, and mesangial cells express Gb3. This, in part, explains the changes observed in D^+HUS glomeruli which are likely due to Stx. Interaction of Stx with these cell types is described next in more detail. Stx may target Gb3-positive glomeruli of the pediatric versus adult kidney, an observation that could explain the higher incidence of D^+HUS among the pediatric population (Lingwood 1994; Chaisri et al. 2001). However, this concept needs to be validated.

3.1.1 Endothelial Cells—Although direct effects of Stx were first described with large vessel endothelial cells, these cells were orders of magnitude less sensitive to concentrations of Stx than microvascular endothelial cells (Obrig et al. 1988, 1993; Louise and Obrig 1994; Ohmi et al. 1998). However, it was noted that only 7% of 55 human umbilical vein endothelial cell cultures, each derived from a different individual, were truly sensitive to the cytotoxic action of Stx (Kaye et al. 1993). The topic of Stx-endothelial interaction has been reviewed recently (Zoja et al. 2001; Muthing et al. 2009; Petruzziello et al. 2009; Karpman et al. 2010; Obrig 2010). It should be noted that the generally accepted pathological description of endothelial damage in D^+HUS is swollen and detached endothelium (Habib 1992; Zoja et al. 2001). While this level of damage appears less severe than cell death,

endothelial cell viability is known to be dependent on attachment to basement membrane (Yannariello-Brown et al. 1988; Hoch et al. 1989; Lalka et al. 1989; Smith et al. 1989; Grant et al. 1990). Given this information, it seems likely that most of the damage to glomerular endothelium is due to direct action of Stx in D^+HUS. However, there are many additional host response factors in the kidney during D^+HUS, some of which are known to interact with endothelium. The health of endothelium is dependent on other cells such as renal glomerular podocytes that produce vascular endothelial cell growth factor (VEGF) essential for endothelial cells (Eremina et al. 2008; Sison et al. 2010). The fact remains that Stx interacts with many different cell types eliciting a myriad of responses (O'Loughlin and Robins-Browne 2001; Karpman et al. 2010; Obrig 2010). Cytokines elicited by bacterial LPS during D^+HUS such as TNF-alpha and IL-1beta, and LPS itself, can induce Gb3 synthesis and increase Stx-sensitivity of endothelial cells (Louise and Obrig 1991, 1992; van de Kar et al. 1992, 1993; Kaye et al. 1993; van Setten et al. 1997a, b; Stricklett et al. 2005).

Responses of endothelial cells to Stx are diverse. Stx was shown to elicit release of chemokines which may be important for development of some aspects of D^+HUS (Zoja et al. 2002; Guessous et al. 2005b). Stx was also shown to decrease prostacyclin synthesis by endothelial cells (Karch et al. 1988). In addition, Stx caused microvascular endothelial cells from human brain or renal glomeruli to apoptose (Kaneko et al. 2001; Pijpers et al. 2001; Ergonul et al. 2003b; Fujii et al. 2008; Psotka et al. 2009). In other cases, Stx activated endothelial cells for increased leukocyte adherence (Zoja et al. 2002; Geelen et al. 2008). As would be expected, some of the direct effects of Stx on endothelial cells result in changes in physiology related to coagulation and thrombosis (Louise and Obrig 1994; van de Kar et al. 1994; Kaye and Obrig 1995; Karpman et al. 2001; Nolasco et al. 2005; Te Loo et al. 2006; Huang et al. 2010). The interaction of Stx and TNF with human renal endothelial cells was shown to elicit tissue factor expression (Nestoridi et al. 2005b). Whether some of these latter events are related to complement activation typical of other thrombotic microangiopathies remains an interesting, but unanswered question (Moake 2009; Thurman et al. 2009; Caprioli et al. 2010; Zipfel et al. 2010). In summary, ample evidence exists demonstrating the central role of endothelial cells in D^+HUS.

3.1.2 Podocytes—Podocytes or visceral epithelial cells are an integral part of the kidney filtration barrier presenting a slit-diaphragm barrier for blood components (Hirschberg et al. 2008; Fogo 2009; Peti-Peterdi and Sipos 2010). Podocytes develop extended foot processes which wrap around the glomerular capillaries and are separated from glomerular endothelium by a basement membrane and glycocalyx. Changes in podocyte physiology are contributing factors in some important renal diseases (Coward et al. 2005; Tryggvason et al. 2006; Henao et al. 2007; Marshall 2007; Collino et al. 2008; D'Agati 2008; Thorner et al. 2008; Quaggin 2009; Clement et al. 2010). Damaged podocytes result in faulty filtration of the blood into the urinary space resulting in increased proteinuria.

Given that podocyte–endothelium interactions are important to both cell types, how does this relate to D^+HUS? One example is that podocytes secrete VEGF that is essential to the nearby endothelial cells (Sison et al. 2010). A Stx-induced decrease in VEGF production by podocytes may add to the proteinuria (Shankland 2006; Eremina et al. 2008; Izzedine et al. 2010). In addition, Stx has been shown to increase endothelin-1 in podocytes that may be deleterious to podocytes (Morigi et al. 2006). An interesting phenomenon is that both CNS neurons and renal podocytes express Gb3, are sensitive to Stx, and exhibit glutamatergic signalling. Podocyte glutamine signalling is important for the function of the filtration barrier (Giardino et al. 2009). While it is has been demonstrated that Stx increases glutamine release in murine neurons, changes in glutamine release have not been studied in glomerular podocytes (Obata et al. 2008; Obata 2010; Obata and Obrig 2010). Important to this review is that human podocyte and glomerular endothelial cells are sensitive to picomolar

concentrations of Stx (Obrig et al. 1993; Psotka et al. 2009). Detailed analyses of Stx effects on glomerular podocytes and endothelial cells have not been reported in animal models of D^+HUS. Stx was detected bound to podocytes in the kidney tissue of an infant who died of D^+HUS (Chaisri et al. 2001). This is in agreement with an earlier report showing that glomeruli from an infant, but not an adult kidney bound Stx (Lingwood 1994). Unfortunately, murine podocyte and endothelial cells do not express Gb3 and are insensitive to Stx, thus placing limits on the use of the murine model of D^+HUS (Rutjes et al. 2002; Psotka et al. 2009). This concept appears to be true for other animal models of D^+HUS including the New Zealand White rabbit.

3.1.3 Mesangial Cells—Expansion of glomerular mesangium occurs in D^+HUS (Shigematsu et al. 1976). Stx binds to and has multiple direct effects on human mesangial cells, in vitro (Robinson et al. 1995; Simon et al. 1998). Although Stx enters these cells to inhibit protein synthesis, mesangial cells are not killed by the toxin (van Setten et al. 1997a, b; Simon et al. 1998). The total importance of Stx interaction with mesangial cells is largely unknown for D^+HUS as very few reports currently exist on this topic. It also appears that basic science knowledge on the normal interaction between mesangial cells and glomerular endothelium or podocytes has lagged behind other areas of kidney research (Vaughan and Quaggin 2008; Picken 2009; Schlondorff and Banas 2009). However, it seems very likely that products elicited in mesangial cells by Stx will be of importance for a complete understanding of D^+HUS pathophysiology.

3.2 Extraglomerular Cells

Sensitivity of renal cells to Stx requires interaction with Gb3 Stx receptors expressed on the plasma membrane. As alluded to above, the distribution of Gb3 among the different tubular epithelial cells of the nephron has not been fully described for human or animal kidneys. However, there is good evidence that some of these specialized cells express Gb3 and are sensitive to Stx (Obrig 2010). The following is a more detailed description of these data. A significant number of immortalized cell lines now exist for in vitro studies of Stx action in the different specialized cells of the nephron (Bens and Vandewalle 2008). At present, it is not clear which animal model of D^+HUS may best represent extraglomerular pathophysiology of D^+HUS in humans (Caprioli et al. 2011).

3.2.1 Proximal Tubule Cells—Proximal tubule cells are the most studied of the renal tubular cell types for D^+HUS. Human proximal tubule cells are sensitive to picomolar concentrations of Stx, in vitro (Hughes et al. 1998a; Sood et al. 2001; Fuller et al. 2011). This high degree of sensitivity to Stx was attributed to ample Gb3 production by human proximal tubule cells, in vitro (Hughes et al. 2002). Interestingly, the StxB (binding) subunit alone also exhibited some responses in this cell type, in vitro (Creydt et al. 2006). Stx induced tissue factor activity on the surface of human proximal tubule cells (Nestoridi et al. 2005a). As there is a role for complement-induced dysfunction of proximal tubules (Buelli et al. 2009), this may also contribute to renal disease in D^+HUS. Low concentrations of Stx induced an increase in TNF-alpha mRNA and release of TNF-alpha from human proximal tubule cells, in vitro (Hughes et al. 1998b). A similar effect was observed in these cells for an increase in IL-1 beta, while Stx synergized with LPS for an increase in IL-6 mRNA (Hughes et al. 1998b). Stx inhibited water absorption by proximal tubule cells in an in vitro assay (Silberstein et al. 2008). Stx caused apoptosis of human proximal tubule cells that could be reversed by silencing the proapototic factor Bak (Karpman et al. 1998; Kodama et al. 1999; Wilson et al. 2005). Finally, it remains to be determined in animal models of D^+HUS whether Stx-induced damage to the filtration barrier results in proteinuria which, in turn, leads to indirect damage to proximal tubules (Zoja et al. 2003; Li et al. 2010; Wu et al. 2010).

3.2.2 Distal Tubule Cells—Mice injected with Stx exhibit specificity for toxin interaction with distal tubules (Rutjes et al. 2002). In some cases the affected cells are described only as cortical or medullary, so interpretation of these data is limited in scope. However, Stx binding to distal tubules has been reported in human renal tissue (Lingwood 1994).

3.2.3 Collecting Duct Cells—Stx targets collecting ducts of mice (Rutjes et al. 2002; Psotka et al. 2009). Renal collecting ducts of mice apoptose in response to intraperitoneally administered Stx (Psotka et al. 2009). Rats given Stx respond with an increased urinary aquaporin-2 level suggesting damage to the AQP2-producing collecting duct cells (Sugatani et al. 2002). Polyuria was reported in both mice and rats given Stx indicating damaged collecting duct cells leading to decreased water reabsorption (Sugatani et al. 2002; Psotka et al. 2009).

3.2.4 Loop of Henle Cells—Very little is known about Stx interaction with epithelial cells of the descending and ascending Loop of Henle in human or animal kidneys. Future in vivo studies of Gb3 co-localization with cell type specific markers will be necessary. Using this approach, preliminary results indicate that anti-Gb3 antibody co-localizes with anti-Tamm Horsfall protein antibody in sections of the murine kidney suggesting that the thick ascending Loop of Henle expresses Gb3 (Obata and Obrig, unpublished data). We also observed co-localization of anti-Gb3 and anti-aquaporin 1 antibodies in medullary sections of human kidney indicating that Gb3 is expressed in the thin descending limb of the Loop of Henle.

In summary, compelling evidence is available supporting a key role of renal microvascular endothelial cells in D^+HUS. However, the fact that human glomerular podocytes are also very sensitive to Stx requires a rethinking of how Stx action leads to renal failure in D^+HUS. In a similar tone, direct effects of Stx on human proximal and collecting duct tubules may explain the dehydration observed in D^+HUS patients. Thus, ample evidence now provides for a rational scheme of how Stx causes renal failure in D^+HUS.

4 LPS Interaction With Cells of the Blood and Resident Renal Cells

4.1 Platelets

LPS is a component of the outer membrane of Gram-negative bacteria which may be released into the circulation leading to endotoxemia and endotoxic shock. It has potent biological activity activating the innate immune response leading to cytokine release (Aderem and Ulevitch 2000). Various studies have shown that LPS may induce platelet activation and aggregation both in vitro (Wachowicz et al. 1998; Saluk-Juszczak et al. 1999) and in vivo (Itoh et al. 1996). This effect may result in consumptive thrombocytopenia (Cicala et al. 1997). Resting platelets must be primed with LPS before interaction with Stx (Viisoreanu et al. 2000; Ståhl et al. 2009). O157LPS binds to platelets via a complex receptor composed of TLR-4 and CD62 (P-selectin) and thus activates them. LPS was also detected on the surface of platelets from HUS patients suggesting that it may activate platelets in the circulation (Ståhl et al. 2006). Thus, as mentioned above, both Stx and O157LPS (Ståhl et al. 2006) are capable of activating platelets and co-stimulation with both virulence factors simultaneously has an additive effect on the formation of platelet-leukocyte complexes expressing tissue factor in whole blood (Karpman et al. 2001; Ghosh et al. 2004; Ståhl et al. 2006, 2009). This effect was further enhanced at increasing shear rates. Co-stimulation of whole blood with Stx and O157LPS also induced an enhanced release of platelets-derived microparticles and tissue factor-coated microparticles, more than each stimulant alone (Ståhl et al. 2009). Thus LPS appears to trigger several mechanisms leading

to a prothrombotic state. O157LPS is at least as potent as other forms of LPS in this respect, if not more (Ståhl et al. 2009).

4.2 Resident Renal Cells

LPS may be important for the development of D^+HUS. Although the amount of LPS in the blood circulation of D^+HUS patients remains undocumented, one would expect a lower level of LPS in D^+HUS than in systemic bacterial infections because D^+HUS is not a bacteremic disease (Tarr et al. 2005; Ahn et al. 2009). The primary receptor for bacterial LPS is toll-like receptor 4 (TLR4) (Beutler and Poltorak 2000; Knotek et al. 2001; Takeuchi and Akira 2001; Yamamoto and Akira 2010). LPS is a more active proinflammatory agent than Stx, and TLR4 receptors are expressed on specific cell types of the kidney. Fortunately, a considerable amount is known about the TLRs in kidneys (Vandewalle 2008; Mkaddem et al. 2010; Pulskens et al. 2010; Batsford et al. 2011; Goncalves et al. 2011). Thus, LPS would be expected to be active in the pathophysiology of D^+HUS. LPS modulates Stx action in cells through induction of Gb3 (Louise and Obrig 1992; Hughes et al. 1998a; 2000; Clayton et al. 2005). In animal models of D^+HUS, administration of LPS either before, during, or after Stx significantly influences the effects of Stx (Barrett et al. 1989; Palermo et al. 2000; Siegler et al. 2001; Clayton et al. 2005; Keepers et al. 2006, 2007). Mice lacking an adequate response to LPS due to a mutation in, or deficiency of, the TLR4 receptor exhibit an increased response to Stx most probably due to decreased bacterial clearance in the gut (Karpman 1997, Calderon Toledo 2008). The action of LPS in animal models of D^+HUS is discussed in more detail below.

5 Inflammatory Responses in the Kidney

D^+HUS exhibits an inflammatory component. Cytokines and chemokines are increased in the blood and kidneys during D^+HUS. The role of these proinflammatory agents in D^+HUS remains to be delineated. The following is a review of data from humans and from animal models of D^+HUS derived from many different laboratories documenting the presence of such agents in D^+HUS.

5.1 Cytokines

The role of TNF-alpha in D^+HUS is controversial. Several reports agree that TNF-alpha can induce Gb3 in some eukaryotic cells, sensitizing the cells to Stx (van de Kar et al. 1992; Louise et al. 1997; van Setten et al. 1997a, b). However, data from a murine model of D^+HUS suggest that TNF-alpha is not an essential contributor to the disease (Wolski et al. 2002). In humans with D^+HUS, TNF-alpha and IL-6 were higher in the urine than in the blood circulation, suggesting localized production of the cytokines (Karpman et al. 1995). These cytokines were not present in the blood and urine of healthy individuals (Karpman et al. 1995). A similar scenario was described for urinary IL-8 in D^+HUS pediatric patients (Inward et al. 1997). Stx directly induced increased release of TNF-alpha from human renal proximal tubule cells, in vitro (Hughes et al. 1998b).

5.2 Chemokines

There is growing evidence of an increase in chemokine synthesis in the kidneys during D^+HUS. In a murine model of D^+HUS, LPS induces C–X–C and C–C chemokines by proximal tubule cells (Keepers et al. 2007; Roche et al. 2007). In homogenates of whole kidney from mice injected with LPS, these chemokines are also increased. In these cases, Stx stabilizes the half-life of chemokine mRNA, further enhancing the total amount of chemokine produced. Chemokines including MCP-1 and IL-8 are increased in urine of D^+HUS patients (van Setten et al. 1998). It is reasonable to expect that individual chemokines may be derived from more than one cell type in D^+HUS. However, it is clear

that inflammatory cells such as monocytes and PMNs are attracted into the kidneys during D^+HUS (Keepers et al. 2006, 2007; Roche et al. 2007). Their relative importance to renal damage in D^+HUS remains to be determined. In the murine model of D^+HUS, it was determined that renal fibrin deposition and the lethal effect of Stx were not affected by the elimination of monocyte/macrophage cell type from these animals (Obrig. et al., unpublished data). Renal chemokines in D^+HUS may act as secondary activators of platelets, along with thrombin or ADP, for renal thrombosis common to this disease (Gear et al. 2001; Gear and Camerini 2003; Guessous et al. 2005b).

5.3 Nitric Oxide

Nitric oxide (NO) is known to exhibit antiplatelet and renal vasodilatory activity (Martin et al. 1986; Radomski et al. 1987). NO was protective in mice against Stx-induced renal toxicity (Dran et al. 2002). However, NO bioavailability was reduced in baboons in response to Stx (Siegler et al. 2005). The role of NO in humans with D^+HUS is not clear. The fact that Stx inhibited NO production by microvascular endothelial cells, in vitro may, in part explain the interaction between Stx and NO in D^+HUS (Te Loo et al. 2006).

6 Thrombosis and Fibrinolysis in D+HUS

A major feature of D^+HUS is the appearance of thrombi in the renal microvasculature. This process involves platelets and endothelial cells. More research is needed to identify additional cell types and inflammatory mediators which also have a role in the thrombosis of D^+HUS. The relative potential of therapeutics for D^+HUS based on targets of thrombosis is made less attractive due to the risk of resulting hemorrhages within the CNS.

6.1 Platelet Activation

Endothelial cells are damaged during HUS. Upon exposure of the subendothelium platelets bind to subendothelial matrix proteins including von Willebrand factor (VWF), fibrinogen and collagen resulting in formation of platelet thrombi. Formation of microthrombi will compromise the blood flow in the microvasculature of the kidney. The initial event in this process is binding of VWF to the platelet membrane receptor glycoprotein 1b (GP1b) allowing a conformational change to occur in the platelet exposing the GPIIb/IIIa receptor on the platelet membrane (Savage et al. 1992). VWF and fibrinogen bind to GPIIb/IIIa which will link one platelet to another, thus forming a clot. Activated platelets release granular contents, such as ADP, thromboxane A2 and thrombin, promoting further activation.

Platelets in HUS appear to be degranulated (Fong and Kaplan 1982; Sassetti et al. 1999) and circulatory levels of β-thromboglobulin (Appiani et al. 1982) and soluble P-selectin (Katayama et al. 1993) are increased. VWF may be secreted from both endothelial cells and platelets and levels are elevated during HUS enabling enhanced formation of thrombi (van de Kar et al. 1994). A decreased multimer size presumably reflects enhanced proteolysis (Tsai et al. 2001) although the activity of ADAMTS13, the vWF cleaving protease, was found to be normal (Tsai et al. 2001). In vitro experiments have shown that Stx induces the release of ultra-large VWF multimers from human endothelial cells and delayed cleavage of VWF by ADAMTS13 (Nolasco et al. 2005), thus promoting the formation of platelet strings attached by ultra-large VWF on endothelial cells. The importance of VWF for promoting platelet aggregation on endothelial cells was illustrated by blocking VWF-platelet specific receptors (Morigi et al. 2001).

There is no consumption of plasma coagulation factors during HUS but prothrombotic markers such as prothrombin 1 + 2 and thrombin–antithrombin III complex are elevated (Nevard et al. 1997; Van Geet et al. 1998) occurring even before patients develop HUS

(Chandler et al. 2002). HUS patients were found to have elevated levels of tissue factor (Kamitsuji et al. 2000) as well as circulating platelet-leukocyte complexes and platelet-derived microparticles, both with deposits of tissue factor and complement on their surface (Ståhl et al. 2009, 2011). These changes were specific for the acute phase of disease, decreasing upon remission. In vitro experiments showed that Stx induced the formation of complexes between platelets and leukocytes, mostly platelets and monocytes. In addition, the toxin induced the release of tissue factor and C3 as well as C9-bearing microparticles, mainly from platelets (Ståhl et al. 2009; Ståhl et al. 2011). Microparticles are procoagulant due to membrane expression of phosphatidylserine (Mallat et al. 2000), an effect that is further enhanced when they are coated with tissue factor. Tissue factor is a transmembrane glycoprotein receptor for coagulation factor VII, leading to conversion of factor X into factor Xa in the extrinsic coagulation pathway (Rao et al. 1986). This will ultimately lead to thrombin generation, resulting in thrombus formation and further platelet activation.

Stx exerts other indirect effects on platelets such as inducing the release of platelet-stimulatory cytokines from monocytes described above (Guessous et al. 2005a). When co-administrated with O157LPS, and especially under high shear rates, the toxin induces pronounced platelet activation (Ståhl et al. 2009). Taken together, platelet activation leading to thrombus formation may occur as a direct effect of bacterial toxin and/or LPS as well as an indirect effect via stimulatory cytokines, in addition to the platelet aggregating events occurring during extensive endothelial cell damage.

6.2 Endothelial Activation

The pathological lesion seen in kidneys during HUS is termed thrombotic microangiopathy and includes the presence of thrombi in glomerular capillaries as well as pronounced endothelial cell swelling and detachment due to subendothelial swelling (Benz and Amann 2009). Damaged endothelium promotes a pro-thrombotic state. Endothelial cell damage presumably occurs before patients develop HUS, as markers of endothelial cell activation (thrombomodulin, soluble vascular cell adhesion molecule and E-selectin) in HUS patients were not found to be higher than in dialysis patients (Nevard et al. 1999).

Stx1 binds to glomerular endothelial cells via the Gb3 receptor and induces a cytotoxic effect (Obrig et al. 1993; van Setten et al. 1997a, b). Stx1 also leads to platelet aggregation on human endothelium (Karpman et al. 2001) particularly on microvascular endothelial cells under conditions of high shear stress (Morigi et al. 2001). Glomerular microvascular endothelial cells pretreated with TNF-α expressed reduced levels of thrombomodulin after incubation with Stx (Fernandez et al. 2003). As thrombomodulin has anti-thrombogenic properties this may promote thrombus formation. Endothelial beta-3-integrin subunit, vitronectin receptor, P-selectin, and PECAM-1 are all involved in Stx-induced platelet aggregation on endothelial cells (Morigi et al. 2001).

In addition to Stx, neutrophils may promote endothelial cell injury during HUS (Forsyth et al. 1989). Stx induces leukocyte adherence to the endothelium (Morigi et al. 1995) as well as their transmigration under flow (Zoja et al. 2002). Activated neutrophils degranulate releasing reactive oxygen species as well as proteases (Fernandez et al. 2005) which may contribute to local cellular injury.

6.3 Fibrinolysis

Under physiological conditions endothelial cells present an antithrombotic surface due to expression of heparan sulphate and thrombomodulin. Heparan sulphate binds antithrombin III which promotes inactivation of the intrinsic coagulation cascade. Thrombomodulin is a glycoprotein receptor on the endothelial surface which binds thrombin thus activating

protein C. Activated protein C and its cofactor, protein S, inactivate factors Va and VIIIa (Lammle and Griffin 1985). Endothelial cells also produce inhibitors of platelet aggregation such as prostacyclin and tissue plasminogen activator (t-PA) (Pearson 2000). Fibrin clots have binding sites for plasminogen and t-PA. t-PA promotes conversion of plasminogen into plasmin. When the fibrinolytic system is activated fibrin is degraded to degradation products, D dimers, by plasmin.

Increased levels of D-dimers, t-PA, t-PA-plasminogen-activator inhibitor type 1 (PAI-1) complex were demonstrated in the early stage of STEC infection even before HUS developed further increasing after the development of HUS (van de Kar et al. 1994; Chandler et al. 2002). Impaired fibrinolysis was demonstrated in patients with STEC-induced HUS (Nevard et al. 1997) which may be due to increased PAI-1 in the circulation (Bergstein et al. 1992). In summary, coagulative aberrations occurring during HUS promote a prothrombotic and hypofibrinolytic state.

7 Development of Acute Renal Failure in D+HUS

The kidney is the main target organ in STEC-mediated HUS. Renal biopsies are not routinely carried out as the diagnosis is made on a clinical basis and patients are usually thrombocytopenic. Histopathological features during STEC-associated HUS are the presence of microthrombi in glomerular capillaries, extensive endothelial damage with occlusion of capillary lumina, deposition of fibrin, mesangiolysis, and mesangial expansion (Shigematsu et al. 1976) as well as extensive tubular apoptosis (Karpman et al. 1998). Severe cases develop acute cortical necrosis affecting most cells in the renal cortex. Bacterial virulence factors may affect the glomerular endothelium, podocytes, mesangial cells as well as tubular cells, as described above. Damage to glomerular endothelium will initiate thrombus formation resulting in multiple microthrombi in glomerular capillaries. Damage to tubular cells will result in electrolyte disturbances, acidosis and decreased urine production. Damaged tubuli may have a secondary deleterious effect on the glomerulus in the same nephron, but the converse is also possible, ischemic injury to the glomerulus will eventually lead to tubular damage within the nephron. Stx was demonstrated in a limited number of human kidneys from patients with HUS, in both glomeruli and tubuli (Uchida et al. 1999; Chaisri et al. 2001). As bacterial toxin may affect both tubular and glomerular cells it is unclear whether the toxin targets certain renal cells preferentially or whether the various cells are affected simultaneously.

In addition to the noxious effect of Stx, presumably in combination with LPS, heme proteins, released during hemolysis, may also have a cytotoxic effect on renal tubular cells and microvascular endothelial cells. This effect was enhanced by Stx in vitro (Bitzan et al. 2004). Recent studies have also raised the possibility that complement-mediated renal injury may occur in STEC-associated HUS. At presentation, patients exhibited activation of the alternative pathway of complement in the circulation (Robson et al. 1992; Thurman et al. 2009; Ståhl et al. 2011). Interestingly, in vitro studies have shown that Stx could induce complement activation and deposition on microvascular endothelial cells under flow (Zoja et al. 2010) as well as activate the alternative pathway in serum (Orth et al. 2009). Complement-mediated renal injury could thus enhance the direct effect of bacterial toxins. Stx may also inhibit the regulatory effect of factor H (Orth et al. 2009) although it is unclear whether this occurs in vivo.

8 Experimental Therapeutics for Renal Disease in D+HUS

Therapeutics are not currently available for the treatment of D^+HUS. Standard supportive care includes maintenance of electrolytes and fluid balance as well as dialysis in more

severe cases. However, the early development and preclinical testing of potential therapeutics has taken place in recent years.

Vaccines are effective to reduce STEC colonization of commercial bovine species (Potter et al. 2004; McNeilly et al. 2010). Vaccines for STEC in humans are less advanced in development and face the test of financial feasibility by pharmaceutical companies. Humanized anti-Stx antibodies have entered the clinical testing phase (Tzipori et al. 2004; Akiyoshi et al. 2005; Dowling et al. 2005; Smith et al. 2006b). Phase I clinical testing of a humanized anti-Stx monoclonal antibody in adult and pediatric subjects demonstrated safety at doses up to 3mg/kg (Lopez et al. 2010). Passive immunization of animals with anti-Stx neutralizing antibodies has demonstrated a moderate degree of protection against challenge with either oral STEC or parenteral Stx (Sauter et al. 2008; Mohawk et al. 2010). The important question to be answered here is whether Stx remains in the blood circulation for a prolonged period of time in D^+HUS patients after the hemorrhagic colitis stage to be available for neutralization by the anti-Stx antibodies. This question remains to be answered. Stx receptor mimics (Lingwood and Mylvaganam 2003; Miura et al. 2006; Watanabe-Takahashi et al. 2010) or inhibitors of Stx enzymatic activity (Wahome et al. 2010) represent different approaches to Stx neutralization in the blood circulation. These agents remain to be approved for clinical testing.

A number of therapeutic strategies have been proposed for late-stage intervention of D^+HUS which target Stx action in the kidneys. Cell permeable small molecules which interfere with Stx trafficking may be effective inhibitors of Stx action (Nishikawa et al. 2006; Sandvig et al. 2009). Others have identified Stx-initiated signal-transduction events in cells as potential targets for therapeutic intervention (Jandhyala et al. 2008). Finally, deleterious host responses to Stx in renal tissue are also potential targets for therapeutics. Examples of these include biological events which lead to coagulation, thrombosis, and changes in the filtration barrier in the kidney. The earlier literature is replete with superficial clinical tests for these targets, none of which showed results sufficient for further testing in properly designed clinical trials. However, some preclinical studies with animal models of D^+HUS have shown promise for late-stage intervention at the kidney level (Nishikawa et al. 2006; Warnier et al. 2006; Roche et al. 2007; Psotka et al. 2009; Jeong et al. 2010).

Development of therapeutics for D^+HUS will depend on measurements of surrogate biomarkers of the disease. These biomarkers are released from damaged cells into the blood circulation or into urine (Rosner 2009). To date, biomarkers specific to D^+HUS are yet to be reported. Urinary neutrophils gelatinase-associated lipocalin (Ngal) has been detected in urine of D^+HUS patients (Trachtman et al. 2006). However, Ngal is less specific as a biomarker of renal injury (Bolignano et al. 2010; Devarajan 2010; Viau et al. 2010). Future application of the Ngal gene reporter mouse to D^+HUS could lead to data more helpful for this application (Paragas et al. 2011). A group of different biomarkers specific to D^+HUS may be required to achieve an acceptable level of clinical utility.

Acknowledgments

This publication was made possible by Grant Numbers AI024431, AI054782, and AI075778 to TGO from the National Institute of Allergy and Infectious Diseases, National Institutes of Health. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. Diana Karpman is supported by grants from The Swedish Research Council (K2010-65X-14008), Torsten and Ragnar Söderberg Foundation, The Fund for Renal Research, Crown Princess Lovisa's Society for Child Care, The Konung Gustaf V:s 80-årsfond, Fanny Ekdahl's Foundation and is the recipient of a clinical-experimental research fellowship from the Royal Swedish Academy of Sciences.

References

Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature. 2000; 406:782–787. [PubMed: 10963608]

Ahn CK, Holt NJ, Tarr PI. Shiga-toxin producing *Escherichia coli* and the hemolytic uremic syndrome: what have we learned in the past 25 years? Adv Exp Med Biol. 2009; 634:1–17. [PubMed: 19280844]

Akiyoshi DE, Rich CM, O'Sullivan-Murphy S, Richard L, Dilo J, Donohue-Rolfe A, Sheoran AS, Chapman-Bonofiglio S, Tzipori S. Characterization of a human monoclonal antibody against Shiga toxin 2 expressed in Chinese hamster ovary cells. Infect Immun. 2005; 73:4054–4061. [PubMed: 15972493]

Appiani AC, Edefonti A, Bettinelli A, Cossu MM, Paracchini ML, Rossi E. The relationship between plasma levels of the factor VIII complex and platelet release products (beta-thromboglobulin and platelet factor 4) in children with the hemolytic-uremic syndrome. Clin Nephrol. 1982; 17:195–199. [PubMed: 6176380]

Arfilli V, Carnicelli D, Rocchi L, Ricci F, Pagliaro P, Tazzari PL, Brigotti M. Shiga toxin 1 and ricin A chain bind to human polymorphonuclear leucocytes through a common receptor. Biochem J. 2010; 432:173–180. [PubMed: 20809900]

Barrett TJ, Potter ME, Wachsmuth IK. Bacterial endotoxin both enhances and inhibits the toxicity of Shiga-like toxin II in rabbits and mice. Infect Immun. 1989; 57:3434–3437. [PubMed: 2680974]

Batsford S, Duermueller U, Seemayer C, Mueller C, Hopfer H, Mihatsch M. Protein level expression of Toll-like receptors 2, 4 and 9 in renal disease. Nephrol Dial Transplant. 2011; 26:1413–1416. [PubMed: 21220755]

Bauer ME, Welch RA. Characterization of an RTX toxin from enterohemorrhagic *Escherichia coli* O157:H7. Infect Immun. 1996; 64:167–175. [PubMed: 8557336]

Bens M, Vandewalle A. Cell models for studying renal physiology. Pflugers Arch. 2008; 457:1–15. [PubMed: 18427833]

Benz K, Amann K. Pathological aspects of membranoproliferative glomerulonephritis (MPGN) and haemolytic uraemic syndrome (HUS)/thrombocytic thrombopenic purpura (TTP). Thromb Haemost. 2009; 101:265–270. [PubMed: 19190808]

Bergstein JM, Riley M, Bang NU. Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. N Engl J Med. 1992; 327:755–759. [PubMed: 1501651]

Betz J, Bielaszewska M, Thies A, Humpf HU, Dreisewerd K, Karch H, Kim KS, Friedrich AW, Muthing J. Shiga toxin glycosphingolipid receptors in microvascular and macrovascular endothelial cells: association with membrane lipid raft microdomains that differ by their stability to cholesterol depletion. J Lipid Res. 2011; 52:618–634. [PubMed: 21252262]

Beutler B, Poltorak A. Positional cloning of Lps, and the general role of toll-like receptors in the innate immune response. Eur Cytokine Netw. 2000; 11:143–152. [PubMed: 10903793]

Bitzan M, Richardson S, Huang C, Boyd B, Petric M, Karmali MA. Evidence that verotoxins (Shiga-like toxins) from *Escherichia coli* bind to P blood group antigens of human erythrocytes in vitro. Infect Immun. 1994; 62:3337–3347. [PubMed: 8039905]

Bitzan M, Bickford BB, Foster GH. Verotoxin (Shiga toxin) sensitizes renal epithelial cells to increased heme toxicity: possible implications for the hemolytic uremic syndrome. J Am Soc Nephrol. 2004; 15:2334–2343. [PubMed: 15339982]

Bolignano D, Coppolino G, Lacquaniti A, Buemi M. From kidney to cardiovascular diseases: NGAL as a biomarker beyond the confines of nephrology. Eur J Clin Invest. 2010; 40:273–276. [PubMed: 20415702]

Boyd B, Lingwood C. Verotoxin receptor glycolipid in human renal tissue. Nephron. 1989; 51:207–210. [PubMed: 2644572]

Brigotti M, Carnicelli D, Ravanelli E, Barbieri S, Ricci F, Bontadini A, Tozzi AE, Scavia G, Caprioli A, Tazzari PL. Interactions between Shiga toxins and human polymorphonuclear leukocytes. J Leukoc Biol. 2008; 84:1019–1027. [PubMed: 18625912]

Brigotti M, Tazzari PL, Ravanelli E, Carnicelli D, Barbieri S, Rocchi L, Arfilli V, Scavia G, Ricci F, Bontadini A, Alfieri RR, Petronini PG, Pecoraro C, Tozzi AE, Caprioli A. Endothelial damage induced by Shiga toxins delivered by neutrophils during transmigration. J Leukoc Biol. 2010; 88:201–210. [PubMed: 20371598]

Brigotti M, Tazzari PL, Ravanelli E, Carnicelli D, Rocchi L, Arfilli V, Scavia G, Minelli F, Ricci F, Pagliaro P, Ferretti AV, Pecoraro C, Paglialonga F, Edefonti A, Procaccino MA, Tozzi AE, Caprioli A. Clinical Relevance of Shiga Toxin Concentrations in the Blood of Patients With Hemolytic Uremic Syndrome. Pediatr Infect Dis J. 2011; 30:486–490. [PubMed: 21164386]

Buelli S, Abbate M, Morigi M, Moioli D, Zanchi C, Noris M, Zoja C, Pusey CD, Zipfel PF, Remuzzi G. Protein load impairs factor H binding promoting complement-dependent dysfunction of proximal tubular cells. Kidney Int. 2009; 75:1050–1059. [PubMed: 19242507]

Calderon Toledo C, Rogers TJ, Svensson M, Tati R, Fischer H, Svanborg C, Karpman D. Shiga toxin-mediated disease in MyD88-deficient mice infected with *Escherichia coli* O157:H7. Am J Pathol. 2008; 173:1428–1439. [PubMed: 18832584]

Caprioli J, Remuzzi G, Noris M. Thrombotic microangiopathies: from animal models to human disease and cure. Contrib Nephrol. 2010; 169:337–350. [PubMed: 21252531]

Caprioli J, Remuzzi G, Noris M. Thrombotic microangiopathies: from animal models to human disease and cure. Contrib Nephrol. 2011; 169:337–350. [PubMed: 21252531]

Chaisri U, Nagata M, Kurazono H, Horie H, Tongtawe P, Hayashi H, Watanabe T, Tapchaisri P, Chongsa-nguan M, Chaicumpa W. Localization of Shiga toxins of enterohaemorrhagic *Escherichia coli* in kidneys of paediatric and geriatric patients with fatal haemolytic uraemic syndrome. Microb Pathog. 2001; 31:59–67. [PubMed: 11453701]

Chandler WL, Jelacic S, Boster DR, Ciol MA, Williams GD, Watkins SL, Igarashi T, Tarr PI. Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. N Engl J Med. 2002; 346:23–32. [PubMed: 11777999]

Cicala C, Santacroce C, Itoh H, Douglas GJ, Page CP. A study on rat platelet responsiveness following intravenous endotoxin administration. Life Sci. 1997; 60:PL31–PL38. [PubMed: 9000123]

Clayton F, Pysher TJ, Lou R, Kohan DE, Denkers ND, Tesh VL, Taylor FB Jr, Siegler RL. Lipopolysaccharide upregulates renal shiga toxin receptors in a primate model of hemolytic uremic syndrome. Am J Nephrol. 2005; 25:536–540. [PubMed: 16179829]

Clement LC, Avila-Casado C, Mace C, Soria E, Bakker WW, Kersten S, Chugh SS. Podocyte-secreted angiopoietin-like-4 mediates proteinuria in glucocorticoid-sensitive nephrotic syndrome. Nat Med. 2010; 17:117–122. [PubMed: 21151138]

Cohen A, Hannigan GE, Williams BR, Lingwood CA. Roles of globotriosyl- and galabiosylceramide in verotoxin binding and high affinity interferon receptor. J Biol Chem. 1987; 262:17088–17091. [PubMed: 2824515]

Cohen A, Madrid-Marina V, Estrov Z, Freedman MH, Lingwood CA, Dosch HM. Expression of glycolipid receptors to Shiga-like toxin on human B lymphocytes: a mechanism for the failure of long-lived antibody response to dysenteric disease. Int Immunol. 1990; 2:1–8. [PubMed: 1965141]

Collino F, Bussolati B, Gerbaudo E, Marozio L, Pelissetto S, Benedetto C, Camussi G. Preeclamptic sera induce nephrin shedding from podocytes through endothelin-1 release by endothelial glomerular cells. Am J Physiol Renal Physiol. 2008; 294:F1185–F1194. [PubMed: 18287402]

Cooling LL, Walker KE, Gille T, Koerner TA. Shiga toxin binds human platelets via globotriaosylceramide (Pk antigen) and a novel platelet glycosphingolipid. Infect Immun. 1998; 66:4355–4366. [PubMed: 9712788]

Coward RJ, Welsh GI, Yang J, Tasman C, Lennon R, Koziell A, Satchell S, Holman GD, Kerjaschki D, Tavare JM, Mathieson PW, Saleem MA. The human glomerular podocyte is a novel target for insulin action. Diabetes. 2005; 54:3095–3102. [PubMed: 16249431]

Creydt VP, Silberstein C, Zotta E, Ibarra C. Cytotoxic effect of Shiga toxin-2 holotoxin and its B subunit on human renal tubular epithelial cells. Microbes Infect. 2006; 8:410–419. [PubMed: 16242986]

D'Agati VD. Podocyte injury in focal segmental glomerulosclerosis: Lessons from animal models (a play in five acts). Kidney Int. 2008; 73:399–406. [PubMed: 17989648]

Del Conde I, Shrimpton CN, Thiagarajan P, Lopez JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood. 2005; 106:1604–1611. [PubMed: 15741221]

Devarajan P. Review: neutrophil gelatinase-associated lipocalin: a troponin-like biomarker for human acute kidney injury. Nephrology (Carlton). 2010; 15:419–428. [PubMed: 20609093]

Dowling TC, Chavaillaz PA, Young DG, Melton-Celsa A, O'Brien A, Thuning-Roberson C, Edelman R, Tacket CO. Phase 1 safety and pharmacokinetic study of chimeric murine-human monoclonal antibody c alpha Stx2 administered intravenously to healthy adult volunteers. Antimicrob Agents Chemother. 2005; 49:1808–1812. [PubMed: 15855500]

Dran GI, Fernandez GC, Rubel CJ, Bermejo E, Gomez S, Meiss R, Isturiz MA, Palermo MS. Protective role of nitric oxide in mice with Shiga toxin-induced hemolytic uremic syndrome. Kidney Int. 2002; 62:1338–1348. [PubMed: 12234304]

Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE. VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med. 2008; 358:1129–1136. [PubMed: 18337603]

Ergonul Z, Clayton F, Fogo AB, Kohan DE. Shiga toxin-1 binding and receptor expression in human kidneys do not change with age. Pediatr Nephrol. 2003a; 18:246–253. [PubMed: 12644917]

Ergonul Z, Hughes AK, Kohan DE. Induction of apoptosis of human brain microvascular endothelial cells by Shiga toxin 1. J Infect Dis. 2003b; 187:154–158. [PubMed: 12508161]

Falguieres T, Mallard F, Baron C, Hanau D, Lingwood C, Goud B, Salamero J, Johannes L. Targeting of Shiga toxin B-subunit to retrograde transport route in association with detergent-resistant membranes. Mol Biol Cell. 2001; 12:2453–2468. [PubMed: 11514628]

Falguieres T, Romer W, Amessou M, Afonso C, Wolf C, Tabet JC, Lamaze C, Johannes L. Functionally different pools of Shiga toxin receptor, globotriaosyl ceramide, in HeLa cells. Febs J. 2006; 273:5205–5218. [PubMed: 17059464]

Fernandez GC, Te Loo MW, van der Velden TJ, van der Heuvel LP, Palermo MS, Monnens LL. Decrease of thrombomodulin contributes to the procoagulant state of endothelium in hemolytic uremic syndrome. Pediatr Nephrol. 2003; 18:1066–1068. [PubMed: 12920633]

Fernandez GC, Gomez SA, Rubel CJ, Bentancor LV, Barrionuevo P, Alduncin M, Grimoldi I, Exeni R, Isturiz MA, Palermo MS. Impaired neutrophils in children with the typical form of hemolytic uremic syndrome. Pediatr Nephrol. 2005; 20:1306–1314. [PubMed: 15940545]

Fernandez GC, Lopez MF, Gomez SA, Ramos MV, Bentancor LV, Fernandez-Brando RJ, Landoni VI, Dran GI, Meiss R, Isturiz MA, Palermo MS. Relevance of neutrophils in the murine model of haemolytic uraemic syndrome: mechanisms involved in Shiga toxin type 2-induced neutrophilia. Clin Exp Immunol. 2006; 146:76–84. [PubMed: 16968401]

Fernandez GC, Gomez SA, Ramos MV, Bentancor LV, Fernandez-Brando RJ, Landoni VI, Lopez L, Ramirez F, Diaz M, Alduncin M, Grimoldi I, Exeni R, Isturiz MA, Palermo MS. The functional state of neutrophils correlates with the severity of renal dysfunction in children with hemolytic uremic syndrome. Pediatr Res. 2007; 61:123–128. [PubMed: 17211153]

Fitzpatrick MM, Shah V, Trompeter RS, Dillon MJ, Barratt TM. Interleukin-8 and polymorphoneutrophil leucocyte activation in hemolytic uremic syndrome of childhood. Kidney Int. 1992; 42:951–956. [PubMed: 1453586]

Fogo AB. The multi-talented podocyte. Nephrol Dial Transplant. 2009; 24:3269–3270. [PubMed: 19720662]

Fong JS, Kaplan BS. Impairment of platelet aggregation in hemolytic uremic syndrome: evidence for platelet "exhaustion". Blood. 1982; 60:564–570. [PubMed: 6213275]

Forsyth KD, Simpson AC, Fitzpatrick MM, Barratt TM, Levinsky RJ. Neutrophil-mediated endothelial injury in haemolytic uraemic syndrome. Lancet. 1989; 2:411–414. [PubMed: 2569599]

Fujii J, Wood K, Matsuda F, Carneiro-Filho BA, Schlegel KH, Yutsudo T, Binnington-Boyd B, Lingwood CA, Obata F, Kim KS, Yoshida S, Obrig T. Shiga toxin 2 causes apoptosis in human brain microvascular endothelial cells via C/EBP homologous protein. Infect Immun. 2008; 76:3679–3689. [PubMed: 18541659]

Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA. Shiga toxin subtypes display dramatic differences in potency. Infect Immun. 2011; 79:1329–1337. [PubMed: 21199911]

Garcia A, Bosques CJ, Wishnok JS, Feng Y, Karalius BJ, Butterton JR, Schauer DB, Rogers AB, Fox JG. Renal injury is a consistent finding in Dutch Belted rabbits experimentally infected with enterohemorrhagic Escherichia coli. J Infect Dis. 2006; 193:1125–1134. [PubMed: 16544253]

Gear AR, Camerini D. Platelet chemokines and chemokine receptors: linking hemostasis, inflammation, and host defense. Microcirculation. 2003; 10:335–350. [PubMed: 12851650]

Gear ARL, Suttitanamongkol D, Viisoreanu D, Polanowska Grabowska RK, Raha S, Camerini D. Adenosine diphosphate strongly potentiates the ability of the chemokines MDC, TARC, and SDF-1 to stimulate platelet function. Blood. 2001; 97:937–945. [PubMed: 11159520]

Geelen JM, van der Velden TJ, Te Loo DM, Boerman OC, van den Heuvel LP, Monnens LA. Lack of specific binding of Shiga-like toxin (verocytotoxin) and non-specific interaction of Shiga-like toxin 2 antibody with human polymorphonuclear leucocytes. Nephrol Dial Transplant. 2007a; 22:749–755. [PubMed: 17127697]

Geelen JM, van der Velden TJ, van den Heuvel LP, Monnens LA. Interactions of Shiga-like toxin with human peripheral blood monocytes. Pediatr Nephrol. 2007b; 22:1181–1187. [PubMed: 17574480]

Geelen J, Valsecchi F, van der Velden T, van den Heuvel L, Monnens L, Morigi M. Shiga-toxin-induced firm adhesion of human leukocytes to endothelium is in part mediated by heparan sulfate. Nephrol Dial Transplant. 2008; 23:3091–3095. [PubMed: 18469308]

Ghosh SA, Polanowska-Grabowska RK, Fujii J, Obrig T, Gear AR. Shiga toxin binds to activated platelets. J Thromb Haemost. 2004; 2:499–506. [PubMed: 15009469]

Giardino L, Armelloni S, Corbelli A, Mattinzoli D, Zennaro C, Guerrot D, Tourrel F, Ikehata M, Li M, Berra S, Carraro M, Messa P, Rastaldi MP. Podocyte glutamatergic signaling contributes to the function of the glomerular filtration barrier. J Am Soc Nephrol. 2009; 20:1929–1940. [PubMed: 19578006]

Goncalves GM, Castoldi A, Braga TT, Camara NO. New roles for innate immune response in acute and chronic kidney injuries. Scand J Immunol. 2011; 73:428–435. [PubMed: 21272051]

Grant DS, Kleinman HK, Martin GR. The role of basement membranes in vascular development. Ann N Y Acad Sci. 1990; 588:61–72. [PubMed: 2192650]

Guessous F, Marcinkiewicz M, Polanowska-Grabowska R, Keepers TR, Obrig T, Gear AR. Shiga toxin 2 and lipopolysaccharide cause monocytic THP-1 cells to release factors which activate platelet function. Thromb Haemost. 2005a; 94:1019–1027. [PubMed: 16366002]

Guessous F, Marcinkiewicz M, Polanowska-Grabowska R, Kongkhum S, Heatherly D, Obrig T, Gear AR. Shiga toxin 2 and lipopolysaccharide induce human microvascular endothelial cells to release chemokines and factors that stimulate platelet function. Infect Immun. 2005b; 73:8306–8316. [PubMed: 16299328]

Habib, R. Pathology of the hemolytic uremic syndrome. In: Kaplan, BS.; Trompeter, RS.; Moake, JL., editors. Thrombotic thrombocytopenic purpura. Decker; New York: 1992. p. 315-353.

Hakomori SI. Cell adhesion/recognition and signal transduction through glycosphingolipid microdomain. Glycoconj J. 2000; 17:143–151. [PubMed: 11201785]

Henao DE, Mathieson PW, Saleem MA, Bueno JC, Cadavid A. A novel renal perspective of preeclampsia: a look from the podocyte. Nephrol Dial Transplant. 2007; 22:1477. [PubMed: 17234666]

Hirschberg R, Wang S, Mitu GM. Functional symbiosis between endothelium and epithelial cells in glomeruli. Cell Tissue Res. 2008; 331:485–493. [PubMed: 17999087]

Hoch J, Jarrell BE, Schneider T, Williams SK. Endothelial cell interactions with native surfaces. Ann Vasc Surg. 1989; 3:153–159. [PubMed: 2765357]

Hoey DE, Sharp L, Currie C, Lingwood CA, Gally DL, Smith DG. Verotoxin 1 binding to intestinal crypt epithelial cells results in localization to lysosomes and abrogation of toxicity. Cell Microbiol. 2003; 5:85–97. [PubMed: 12580945]

Huang J, Motto DG, Bundle DR, Sadler JE. Shiga toxin B subunits induce VWF secretion by human endothelial cells and thrombotic microangiopathy in ADAMTS13-deficient mice. Blood. 2010; 116:3653–3659. [PubMed: 20644116]

Hughes AK, Stricklett PK, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. Kidney Int. 1998a; 54:426–437. [PubMed: 9690209]

Hughes AK, Stricklett PK, Kohan DE. Shiga toxin-1 regulation of cytokine production by human proximal tubule cells. Kidney Int. 1998b; 54:1093–1106. [PubMed: 9767525]

Hughes AK, Stricklett PK, Schmid D, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human glomerular epithelial cells. Kidney Int. 2000; 57:2350–2359. [PubMed: 10844605]

Hughes AK, Ergonul Z, Stricklett PK, Kohan DE. Molecular basis for high renal cell sensitivity to the cytotoxic effects of shigatoxin-1: upregulation of globotriaosylceramide expression. J Am Soc Nephrol. 2002; 13:2239–2245. [PubMed: 12191968]

Inward CD, Varagunam M, Adu D, Milford DV, Taylor CM. Cytokines in haemolytic uraemic syndrome associated with verocytotoxin-producing *Escherichia coli* infection. Arch Dis Child. 1997; 77:145–147. [PubMed: 9301354]

Itoh H, Cicala C, Douglas GJ, Page CP. Platelet accumulation induced by bacterial endotoxin in rats. Thromb Res. 1996; 83:405–419. [PubMed: 8885136]

Izzedine H, Massard C, Spano JP, Goldwasser F, Khayat D, Soria JC. VEGF signalling inhibition-induced proteinuria: Mechanisms, significance and management. Eur J Cancer. 2010; 46:439–448. [PubMed: 20006922]

Jandhyala DM, Ahluwalia A, Obrig T, Thorpe CM. ZAK: a MAP3Kinase that transduces Shiga toxin- and ricin-induced proinflammatory cytokine expression. Cell Microbiol. 2008; 10:1468–1477. [PubMed: 18331592]

Jelacic S, Wobbe CL, Boster DR, Ciol MA, Watkins SL, Tarr PI, Stapleton AE. ABO and P1 blood group antigen expression and stx genotype and outcome of childhood *Escherichia coli* O157:H7 infections. J Infect Dis. 2002; 185:214–219. [PubMed: 11807695]

Jeong KI, Chapman-Bonofiglio S, Singh P, Lee J, Tzipori S, Sheoran AS. In vitro and in vivo protective efficacies of antibodies that neutralize the RNA N-glycosidase activity of Shiga toxin 2. BMC Immunol. 2010; 11:16. [PubMed: 20334660]

Johannes L, Romer W. Shiga toxins—from cell biology to biomedical applications. Nat Rev Microbiol. 2010; 8:105–116. [PubMed: 20023663]

Kamitsuji H, Nonami K, Murakami T, Ishikawa N, Nakayama A, Umeki Y. Elevated tissue factor circulating levels in children with hemolytic uremic syndrome caused by verotoxin-producing *E. coli*. Clin Nephrol. 2000; 53:319–324. [PubMed: 11305804]

Kaneko K, Kiyokawa N, Ohtomo Y, Nagaoka R, Yamashiro Y, Taguchi T, Mori T, Fujimoto J, Takeda T. Apoptosis of renal tubular cells in Shiga-toxin-mediated hemolytic uremic syndrome. Nephron. 2001; 87:182–185. [PubMed: 11244315]

Karch H, Bitzan M, Pietsch R, Stenger KO, von Wulffen H, Heesemann J, Dusing R. Purified verotoxins of *Escherichia coli* O157:H7 decrease prostacyclin synthesis by endothelial cells. Microb Pathog. 1988; 5:215–221. [PubMed: 3063914]

Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol. 2005; 295:405–418. [PubMed: 16238016]

Karpman D, Andreasson A, Thysell H, Kaplan BS, Svanborg C. Cytokines in childhood hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Pediatr Nephrol. 1995; 9:694–699. [PubMed: 8747107]

Karpman D, Connell H, Svensson M, Scheutz F, Alm P, Svanborg C. The role of lipopolysaccharide and Shiga-like toxin in a mouse model of *Escherichia coli* O157:H7 infection. J Infect Dis. 1997; 175:611–620. [PubMed: 9041333]

Karpman D, Hakansson A, Perez MT, Isaksson C, Carlemalm E, Caprioli A, Svanborg C. Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. Infect Immun. 1998; 66:636–644. [PubMed: 9453620]

Karpman D, Papadopoulou D, Nilsson K, Sjogren AC, Mikaelsson C, Lethagen S. Platelet activation by Shiga toxin and circulatory factors as a pathogenetic mechanism in the hemolytic uremic syndrome. Blood. 2001; 97:3100–3108. [PubMed: 11342436]

Karpman D, Sartz L, Johnson S. Pathophysiology of typical hemolytic uremic syndrome. Semin Thromb Hemost. 2010; 36:575–585. [PubMed: 20865634]

Katayama M, Handa M, Araki Y, Ambo H, Kawai Y, Watanabe K, Ikeda Y. Soluble P-selectin is present in normal circulation and its plasma level is elevated in patients with thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome. Br J Haematol. 1993; 84:702–710. [PubMed: 7692937]

Kaye SA, Obrig TG. Effect of TNF-alpha, Shiga toxin and calcium ionophore on Weibel-Palade body content of endothelial cells: possible implications for the hemolytic uremic syndrome. Thromb Res. 1995; 79:415–421. [PubMed: 7482445]

Kaye SA, Louise CB, Boyd B, Lingwood CA, Obrig TG. Shiga toxin-associated hemolytic uremic syndrome: interleukin-1 beta enhancement of Shiga toxin cytotoxicity toward human vascular endothelial cells in vitro. Infect Immun. 1993; 61:3886–3891. [PubMed: 8359910]

Keepers TR, Psotka MA, Gross LK, Obrig TG. A murine model of HUS: shiga toxin with lipopolysaccharide mimics the renal damage and physiologic response of human disease. J Am Soc Nephrol. 2006; 17:3404–3414. [PubMed: 17082244]

Keepers TR, Gross LK, Obrig TG. Monocyte chemoattractant protein 1, macrophage inflammatory protein 1 alpha, and RANTES recruit macrophages to the kidney in a mouse model of hemolytic-uremic syndrome. Infect Immun. 2007; 75:1229–1236. [PubMed: 17220320]

Khan F, Proulx F, Lingwood CA. Detergent-resistant globotriaosyl ceramide may define verotoxin/glomeruli-restricted hemolytic uremic syndrome pathology. Kidney Int. 2009; 75:1209–1216. [PubMed: 19212418]

Knotek M, Rogachev B, Wang W, Ecder T, Melnikov V, Gengaro PE, Esson M, Edelstein CL, Dinarello CA, Schrier RW. Endotoxemic renal failure in mice: role of tumor necrosis factor independent of inducible nitric oxide synthase. Kidney Int. 2001; 59:2243–2249. [PubMed: 11380827]

Kodama T, Nagayama K, Yamada K, Ohba Y, Akeda Y, Honda T. Induction of apoptosis in human renal proximal tubular epithelial cells by *Escherichia coli* verocytotoxin 1 in vitro. Med Microbiol Immunol. 1999; 188:73–78. [PubMed: 10753058]

Kurioka T, Yunou Y, Kita E. Enhancement of susceptibility to Shiga toxin-producing *Escherichia coli* O157:H7 by protein calorie malnutrition in mice. Infect Immun. 1998; 66:1726–1734. [PubMed: 9529103]

Lalka SG, Oelker LM, Malone JM, Duhamel RC, Kevorkian MA, Raper BA, Nixon JC, Etchberger KJ, Dalsing MC, Cikrit DF, et al. Acellular vascular matrix: a natural endothelial cell substrate. Ann Vasc Surg. 1989; 3:108–117. [PubMed: 2765354]

Lammle B, Griffin JH. Formation of the fibrin clot: the balance of procoagulant and inhibitory factors. Clin Haematol. 1985; 14:281–342. [PubMed: 2994929]

Li X, Pabla N, Wei Q, Dong G, Messing RO, Wang CY, Dong Z. PKC-delta promotes renal tubular cell apoptosis associated with proteinuria. J Am Soc Nephrol. 2010; 21:1115–1124. [PubMed: 20395372]

Lindberg AA, Brown JE, Stromberg N, Westling-Ryd M, Schultz JE, Karlsson KA. Identification of the carbohydrate receptor for Shiga toxin produced by Shigella dysenteriae type 1. J Biol Chem. 1987; 262:1779–1785. [PubMed: 3543013]

Lingwood CA. Verotoxins and their glycolipid receptors. Adv Lipid Res. 1993; 25:189–211. [PubMed: 8368148]

Lingwood CA. Verotoxin-binding in human renal sections. Nephron. 1994; 66:21–28. [PubMed: 8107950]

Lingwood CA. Role of verotoxin receptors in pathogenesis. Trends Microbiol. 1996; 4:147–153. [PubMed: 8728608]

Lingwood CA, Mylvaganam M. Lipid modulation of glycosphingolipid (GSL) receptors: soluble GSL mimics provide new probes of GSL receptor function. Methods Enzymol. 2003; 363:264–283. [PubMed: 14579581]

Lingwood CA, Law H, Richardson S, Petric M, Brunton JL, De Grandis S, Karmali M. Glycolipid binding of purified and recombinant Escherichia coli produced verotoxin in vitro. J Biol Chem. 1987; 262:8834–8839. [PubMed: 3298243]

Lingwood CA, Binnington B, Manis A, Branch DR. Globotriaosyl ceramide receptor function—where membrane structure and pathology intersect. FEBS Lett. 2010a; 584:1879–1886. [PubMed: 19948172]

Lingwood CA, Manis A, Mahfoud R, Khan F, Binnington B, Mylvaganam M. New aspects of the regulation of glycosphingolipid receptor function. Chem Phys Lipids. 2010b; 163:27–35. [PubMed: 19781539]

Liu J, Akahoshi T, Sasahana T, Kitasato H, Namai R, Sasaki T, Inoue M, Kondo H. Inhibition of neutrophil apoptosis by verotoxin 2 derived from *Escherichia coli* O157:H7. Infect Immun. 1999a; 67:6203–6205. [PubMed: 10531291]

Liu XH, Lingwood CA, Ray PE. Recruitment of renal tubular epithelial cells expressing verotoxin-1 (Stx1) receptors in HIV-1 transgenic mice with renal disease. Kidney Int. 1999b; 55:554–561. [PubMed: 9987079]

Lopez EL, Contrini MM, Glatstein E, Gonzalez Ayala S, Santoro R, Allende D, Ezcurra G, Teplitz E, Koyama T, Matsumoto Y, Sato H, Sakai K, Hoshide S, Komoriya K, Morita T, Harning R, Brookman S. Safety and pharmacokinetics of urtoxazumab, a humanized monoclonal antibody, against Shiga-like toxin 2 in healthy adults and in pediatric patients infected with Shiga-like toxin-producing Escherichia coli. Antimicrob Agents Chemother. 2010; 54:239–243. [PubMed: 19822704]

Louise CB, Obrig TG. Shiga toxin-associated hemolytic-uremic syndrome: combined cytotoxic effects of Shiga toxin, interleukin-1 beta, and tumor necrosis factor alpha on human vascular endothelial cells in vitro. Infect Immun. 1991; 59:4173–4179. [PubMed: 1937774]

Louise CB, Obrig TG. Shiga toxin-associated hemolytic uremic syndrome: combined cytotoxic effects of shiga toxin and lipopolysaccharide (endotoxin) on human vascular endothelial cells in vitro. Infect Immun. 1992; 60:1536–1543. [PubMed: 1548077]

Louise CB, Obrig TG. Human renal microvascular endothelial cells as a potential target in the development of the hemolytic uremic syndrome as related to fibrinolysis factor expression, in vitro. Microvasc Res. 1994; 47:377–387. [PubMed: 8084301]

Louise CB, Tran MC, Obrig TG. Sensitization of human umbilical vein endothelial cells to Shiga toxin: involvement of protein kinase C and NF-kappaB. Infect Immun. 1997; 65:3337–3344. [PubMed: 9234795]

Mahfoud R, Manis A, Binnington B, Ackerley C, Lingwood CA. A major fraction of glycosphingolipids in model and cellular cholesterol containing membranes are undetectable by their binding proteins. J Biol Chem. 2010; 285:36049–36059. [PubMed: 20716521]

Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation. 2000; 101:841–843. [PubMed: 10694520]

Marshall SM. The podocyte: a potential therapeutic target in diabetic nephropathy? Curr Pharm Des. 2007; 13:2713–2720. [PubMed: 17897015]

Martin W, Furchgott RF, Villani GM, Jothianandan D. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. J Pharmacol Exp Ther. 1986; 237:529–538. [PubMed: 3009791]

McCluer RH, Williams MA, Gross SK, Meisler MH. Testosterone effects on the induction and urinary excretion of mouse kidney glycosphingolipids associated with lysosomes. J Biol Chem. 1981; 256:13112–13120. [PubMed: 7309755]

McNeilly TN, Mitchell MC, Rosser T, McAteer S, Low JC, Smith DG, Huntley JF, Mahajan A, Gally DL. Immunization of cattle with a combination of purified intimin-531, EspA and Tir significantly reduces shedding of *Escherichia coli* O157:H7 following oral challenge. Vaccine. 2010; 28:1422–1428. [PubMed: 19903545]

Melton-Celsa AR, O'Brien AD. Animal models for STEC-mediated disease. Methods Mol Med. 2003; 73:291–305. [PubMed: 12375438]

Miura Y, Sakaki A, Kamihira M, Iijima S, Kobayashi K. A globotriaosylceramide (Gb3Cer) mimic peptide isolated from phage display library expressed strong neutralization to Shiga toxins. Biochim Biophys Acta. 2006; 1760:883–889. [PubMed: 16713681]

Mkaddem SB, Bens M, Vandewalle A. Differential activation of Toll-like receptor-mediated apoptosis induced by hypoxia. Oncotarget. 2010; 1:741–750. [PubMed: 21321383]

Moake J. Thrombotic thrombocytopenia purpura (TTP) and other thrombotic microangiopathies. Best Pract Res Clin Haematol. 2009; 22:567–576. [PubMed: 19959109]

Mohawk KL, Melton-Celsa AR, Robinson CM, O'Brien AD. Neutralizing antibodies to Shiga toxin type 2 (Stx2) reduce colonization of mice by Stx2-expressing *Escherichia coli* O157:H7. Vaccine. 2010; 28:4777–4785. [PubMed: 20472033]

Morigi M, Micheletti G, Figliuzzi M, Imberti B, Karmali MA, Remuzzi A, Remuzzi G, Zoja C. Verotoxin-1 promotes leukocyte adhesion to cultured endothelial cells under physiologic flow conditions. Blood. 1995; 86:4553–4558. [PubMed: 8541545]

Morigi M, Galbusera M, Binda E, Imberti B, Gastoldi S, Remuzzi A, Zoja C, Remuzzi G. Verotoxin-1-induced up-regulation of adhesive molecules renders microvascular endothelial cells thrombogenic at high shear stress. Blood. 2001; 98:1828–1835. [PubMed: 11535517]

Morigi M, Buelli S, Zanchi C, Longaretti L, Macconi D, Benigni A, Moioli D, Remuzzi G, Zoja C. Shigatoxin-induced endothelin-1 expression in cultured podocytes autocrinally mediates actin remodeling. Am J Pathol. 2006; 169:1965–1975. [PubMed: 17148661]

Murata K, Higuchi T, Takada K, Oida K, Horie S, Ishii H. Verotoxin-1 stimulation of macrophage-like THP-1 cells up-regulates tissue factor expression through activation of c-Yes tyrosine kinase: possible signal transduction in tissue factor up-regulation. Biochim Biophys Acta. 2006; 1762:835–843. [PubMed: 16930953]

Muthing J, Schweppe CH, Karch H, Friedrich AW. Shiga toxins, glycosphingolipid diversity, and endothelial cell injury. Thromb Haemost. 2009; 101:252–264. [PubMed: 19190807]

Nestoridi E, Kushak RI, Duguerre D, Grabowski EF, Ingelfinger JR. Up-regulation of tissue factor activity on human proximal tubular epithelial cells in response to Shiga toxin. Kidney Int. 2005a; 67:2254–2266. [PubMed: 15882267]

Nestoridi E, Tsukurov O, Kushak RI, Ingelfinger JR, Grabowski EF. Shiga toxin enhances functional tissue factor on human glomerular endothelial cells: implications for the pathophysiology of hemolytic uremic syndrome. J Thromb Haemost. 2005b; 3:752–762. [PubMed: 15842359]

Nevard CH, Jurd KM, Lane DA, Philippou H, Haycock GB, Hunt BJ. Activation of coagulation and fibrinolysis in childhood diarrhoea-associated haemolytic uraemic syndrome. Thromb Haemost. 1997; 78:1450–1455. [PubMed: 9423793]

Nevard CH, Blann AD, Jurd KM, Haycock GB, Hunt BJ. Markers of endothelial cell activation and injury in childhood haemolytic uraemic syndrome. Pediatr Nephrol. 1999; 13:487–492. [PubMed: 10452276]

Newburg DS, Chaturvedi P, Lopez EL, Devoto S, Fayad A, Cleary TG. Susceptibility to hemolytic-uremic syndrome relates to erythrocyte glycosphingolipid patterns. J Infect Dis. 1993; 168:476–479. [PubMed: 8335990]

Nishikawa K, Watanabe M, Kita E, Igai K, Omata K, Yaffe MB, Natori Y. A multivalent peptide library approach identifies a novel Shiga toxin inhibitor that induces aberrant cellular transport of the toxin. Faseb J. 2006; 20:2597–2599. [PubMed: 17065223]

Nolasco LH, Turner NA, Bernardo A, Tao Z, Cleary TG, Dong JF, Moake JL. Hemolytic uremic syndrome-associated Shiga toxins promote endothelial-cell secretion and impair ADAMTS13 cleavage of unusually large von Willebrand factor multimers. Blood. 2005; 106:4199–4209. [PubMed: 16131569]

Obata F. Influence of *Escherichia coli* shiga toxin on the mammalian central nervous system. Adv Appl Microbiol. 2010; 71:1–19. [PubMed: 20378049]

Obata F, Obrig T. Distribution of Gb(3) immunoreactivity in the mouse central nervous system. Toxins (Basel). 2010; 2:1997–2006. [PubMed: 20725533]

Obata F, Tohyama K, Bonev AD, Kolling GL, Keepers TR, Gross LK, Nelson MT, Sato S, Obrig TG. Shiga toxin 2 affects the central nervous system through receptor globotriaosylceramide localized to neurons. J Infect Dis. 2008; 198:1398–1406. [PubMed: 18754742]

Obrig TG. *Escherichia coli* shiga toxin mechanisms of action in renal disease. Toxins (Basel). 2010; 2:2769–2794. [PubMed: 21297888]

Obrig TG, Del Vecchio PJ, Brown JE, Moran TP, Rowland BM, Judge TK, Rothman SW. Direct cytotoxic action of Shiga toxin on human vascular endothelial cells. Infect Immun. 1988; 56:2373–2378. [PubMed: 3044997]

Obrig TG, Louise CB, Lingwood CA, Boyd B, Barley-Maloney L, Daniel TO. Endothelial heterogeneity in Shiga toxin receptors and responses. J Biol Chem. 1993; 268:15484–15488. [PubMed: 8340376]

Ohmi K, Kiyokawa N, Takeda T, Fujimoto J. Human microvascular endothelial cells are strongly sensitive to Shiga toxins. Biochem Biophys Res Commun. 1998; 251:137–141. [PubMed: 9790920]

Okuda T, Tokuda N, Numata S, Ito M, Ohta M, Kawamura K, Wiels J, Urano T, Tajima O, Furukawa K. Targeted disruption of Gb3/CD77 synthase gene resulted in the complete deletion of globo-series glycosphingolipids and loss of sensitivity to verotoxins. J Biol Chem. 2006; 281:10230–10235. [PubMed: 16476743]

O'Loughlin EV, Robins-Browne RM. Effect of shiga toxin and shiga-like toxins on eukaryotic cells. Microbes Infect. 2001; 3:493–507. [PubMed: 11377211]

Orth D, Khan AB, Naim A, Grif K, Brockmeyer J, Karch H, Joannidis M, Clark SJ, Day AJ, Fidanzi S, Stoiber H, Dierich MP, Zimmerhackl LB, Wurzner R. Shiga toxin activates complement and binds factor H: evidence for an active role of complement in hemolytic uremic syndrome. J Immunol. 2009; 182:6394–6400. [PubMed: 19414792]

Paixao-Cavalcante D, Botto M, Cook HT, Pickering MC. Shiga toxin-2 results in renal tubular injury but not thrombotic microangiopathy in heterozygous factor H-deficient mice. Clin Exp Immunol. 2009; 155:339–347. [PubMed: 19040606]

Palermo M, Alves-Rosa F, Rubel C, Fernandez GC, Fernandez-Alonso G, Alberto F, Rivas M, Isturiz M. Pretreatment of mice with lipopolysaccharide (LPS) or IL-1beta exerts dose-dependent opposite effects on Shiga toxin-2 lethality. Clin Exp Immunol. 2000; 119:77–83. [PubMed: 10606967]

Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, Viltard M, Yu W, Forster CS, Gong G, Liu Y, Kulkarni R, Mori K, Kalandadze A, Ratner AJ, Devarajan P, Landry DW, D'Agati V, Lin CS, Barasch J. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat Med. 2011; 17:216–222. [PubMed: 21240264]

Pearson JD. Normal endothelial cell function. Lupus. 2000; 9:183–188. [PubMed: 10805485]

Peti-Peterdi J, Sipos A. A high-powered view of the filtration barrier. J Am Soc Nephrol. 2010; 21:1835–1841. [PubMed: 20576805]

Petruzziello TN, Mawji IA, Khan M, Marsden PA. Verotoxin biology: molecular events in vascular endothelial injury. Kidney Int Suppl. 2009; 112:S17–S19. [PubMed: 19180125]

Picken MM. The role of mesangial homeostasis in glomerular injury progression: hope for mesangial sclerosis reversal. Kidney Int. 2009; 75:574–576. [PubMed: 19247379]

Pijpers AH, van Setten PA, van den Heuvel LP, Assmann KJ, Dijkman HB, Pennings AH, Monnens LA, van Hinsbergh VW. Verocytotoxin-induced apoptosis of human microvascular endothelial cells. J Am Soc Nephrol. 2001; 12:767–778. [PubMed: 11274238]

Potter AA, Klashinsky S, Li Y, Frey E, Townsend H, Rogan D, Erickson G, Hinkley S, Klopfenstein T, Moxley RA, Smith DR, Finlay BB. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine. 2004; 22:362–369. [PubMed: 14670317]

Psotka MA, Obata F, Kolling GL, Gross LK, Saleem MA, Satchell SC, Mathieson PW, Obrig TG. Shiga toxin 2 targets the murine renal collecting duct epithelium. Infect Immun. 2009; 77:959–969. [PubMed: 19124603]

Pulskens WP, Rampanelli E, Teske GJ, Butter LM, Claessen N, Luirink IK, van der Poll T, Florquin S, Leemans JC. TLR4 promotes fibrosis but attenuates tubular damage in progressive renal injury. J Am Soc Nephrol. 2010; 21:1299–1308. [PubMed: 20595685]

Quaggin SE. Genetic susceptibility to HIV-associated nephropathy. J Clin Invest. 2009; 119:1085–1089. [PubMed: 19422099]

Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet. 1987; 2:1057–1058. [PubMed: 2889967]

Rao LV, Rapaport SI, Bajaj SP. Activation of human factor VII in the initiation of tissue factor-dependent coagulation. Blood. 1986; 68:685–691. [PubMed: 3488776]

Robinson LA, Hurley RM, Lingwood C, Matsell DG. *Escherichia coli* verotoxin binding to human paediatric glomerular mesangial cells. Pediatr Nephrol. 1995; 9:700–704. [PubMed: 8747108]

Robson WL, Fick GH, Wilson PC. Prognostic factors in typical postdiarrhea hemolytic-uremic syndrome. Child Nephrol Urol. 1988; 9:203–207. [PubMed: 3255484]

Robson WL, Leung AK, Fick GH, McKenna AI. Hypocomplementemia and leukocytosis in diarrhea-associated hemolytic uremic syndrome. Nephron. 1992; 62:296–299. [PubMed: 1436342]

Roche JK, Keepers TR, Gross LK, Seaner RM, Obrig TG. CXCL1/KC and CXCL2/MIP-2 are critical effectors and potential targets for therapy of *Escherichia coli* O157:H7-associated renal inflammation. Am J Pathol. 2007; 170:526–537. [PubMed: 17255321]

Rosner MH. Urinary biomarkers for the detection of renal injury. Adv Clin Chem. 2009; 49:73–97. [PubMed: 19947356]

Rutjes NW, Binnington BA, Smith CR, Maloney MD, Lingwood CA. Differential tissue targeting and pathogenesis of verotoxins 1 and 2 in the mouse animal model. Kidney Int. 2002; 62:832–845. [PubMed: 12164865]

Saluk-Juszczak J, Wachowicz B, Kaca W. Stimulatory effects of endotoxin on the platelet secretory process. Microbios. 1999; 99:45–53. [PubMed: 10624010]

Sandvig, K.; Bergan, J.; Dyve, AB.; Skotland, T.; Torgersen, ML. Endocytosis and retrograde transport of Shiga toxin. Toxicon: 2009.

Sassetti B, Vizcarguenaga MI, Zanaro NL, Silva MV, Kordich L, Florentini L, Diaz M, Vitacco M, Sanchez Avalos JC. Hemolytic uremic syndrome in children: platelet aggregation and membrane glycoproteins. J Pediatr Hematol Oncol. 1999; 21:123–128. [PubMed: 10206458]

Sauter KA, Melton-Celsa AR, Larkin K, Troxell ML, O'Brien AD, Magun BE. Mouse model of hemolytic-uremic syndrome caused by endotoxin-free Shiga toxin 2 (Stx2) and protection from lethal outcome by anti-Stx2 antibody. Infect Immun. 2008; 76:4469–4478. [PubMed: 18694970]

Savage B, Shattil SJ, Ruggeri ZM. Modulation of platelet function through adhesion receptors. A dual role for glycoprotein IIb–IIIa (integrin alpha IIb beta 3) mediated by fibrinogen and glycoprotein Ib-von Willebrand factor. J Biol Chem. 1992; 267:11300–11306. [PubMed: 1597464]

Schlondorff D, Banas B. The mesangial cell revisited: no cell is an island. J Am Soc Nephrol. 2009; 20:1179–1187. [PubMed: 19470685]

Schmidt H, Karch H, Beutin L. The large-sized plasmids of enterohemorrhagic *Escherichia coli* O157 strains encode hemolysins which are presumably members of the *E. coli* alpha-hemolysin family. FEMS Microbiol Lett. 1994; 117:189–196. [PubMed: 8181722]

Shankland SJ. The podocyte's response to injury: role in proteinuria and glomerulosclerosis. Kidney Int. 2006; 69:2131–2147. [PubMed: 16688120]

Shayman JA, Radin NS. Structure and function of renal glycosphingolipids. Am J Physiol. 1991; 260:F291–F302. [PubMed: 2000947]

Shigematsu H, Dikman SH, Churg J, Grishman E, Duffy JL. Mesangial involvement in hemolytic-uremic syndrome. A light and electron microscopic study. Am J Pathol. 1976; 85:349–362. [PubMed: 998726]

Siegler RL, Pysher TJ, Lou R, Tesh VL, Taylor FB Jr. Response to shiga toxin-1, with and without lipopolysaccharide, in a primate model of hemolytic uremic syndrome. Am J Nephrol. 2001; 21:420–425. [PubMed: 11684808]

Siegler RL, Obrig TG, Pysher TJ, Tesh VL, Denkers ND, Taylor FB. Response to shiga toxin 1 and 2 in a baboon model of hemolytic uremic syndrome. Pediatr Nephrol. 2003; 18:92–96. [PubMed: 12579394]

Siegler RL, Pysher TJ, Tesh VL, Noris M, Cassis P, Taylor FB Jr. Reduced nitric oxide bioavailability in a baboon model of Shiga toxin mediated hemolytic uremic syndrome (HUS). Ren Fail. 2005; 27:635–641. [PubMed: 16153006]

Silberstein C, Pistone Creydt V, Gerhardt E, Nunez P, Ibarra C. Inhibition of water absorption in human proximal tubular epithelial cells in response to shiga toxin-2. Pediatr Nephrol. 2008; 23:1981–1990. [PubMed: 18607643]

Simon M, Cleary TG, Hernandez JD, Abboud HE. Shiga toxin 1 elicits diverse biologic responses in mesangial cells. Kidney Int. 1998; 54:1117–1127. [PubMed: 9767527]

Sison K, Eremina V, Baelde H, Min W, Hirashima M, Fantus IG, Quaggin SE. Glomerular structure and function require paracrine, not autocrine, VEGF-VEGFR-2 signaling. J Am Soc Nephrol. 2010; 21:1691–1701. [PubMed: 20688931]

Smith PS, Fanning JC, Aarons I. The structure of the normal human glomerular basement membrane. Ultrastructural localization of type IV collagen and laminin. Pathology. 1989; 21:254–258. [PubMed: 2633114]

Smith DC, Sillence DJ, Falguieres T, Jarvis RM, Johannes L, Lord JM, Platt FM, Roberts LM. The association of Shiga-like toxin with detergent-resistant membranes is modulated by glucosylceramide and is an essential requirement in the endoplasmic reticulum for a cytotoxic effect. Mol Biol Cell. 2006a; 17:1375–1387. [PubMed: 16381816]

Smith MJ, Teel LD, Carvalho HM, Melton-Celsa AR, O'Brien AD. Development of a hybrid Shiga holotoxoid vaccine to elicit heterologous protection against Shiga toxins types 1 and 2. Vaccine. 2006b; 24:4122–4129. [PubMed: 16551486]

Sood A, Mathew R, Trachtman H. Cytoprotective effect of curcumin in human proximal tubule epithelial cells exposed to shiga toxin. Biochem Biophys Res Commun. 2001; 283:36–41. [PubMed: 11322764]

Spitalnik PF, Spitalnik SL. The P blood group system: biochemical, serological, and clinical aspects. Transfus Med Rev. 1995; 9:110–122. [PubMed: 7795329]

Ståhl AL, Svensson M, Morgelin M, Svanborg C, Tarr PI, Mooney JC, Watkins SL, Johnson R, Karpman D. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets through TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. Blood. 2006; 108:167–176. [PubMed: 16514062]

Ståhl AL, Sartz L, Nelsson A, Bekassy ZD, Karpman D. Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. PLoS One. 2009; 4:e6990. [PubMed: 19750223]

Ståhl AL, Sartz L, Karpman D. Complement activation on platelet-leukocyte complexes and microparticles in enterohemorrhagic *Escherichia coli*-induced hemolytic uremic syndrome. Blood. 2011; 117:5503–5513. [PubMed: 21447825]

Stearns-Kurosawa DJ, Collins V, Freeman S, Tesh VL, Kurosawa S. Distinct physiologic and inflammatory responses elicited in baboons after challenge with Shiga toxin type 1 or 2 from enterohemorrhagic Escherichia coli. Infect Immun. 2010; 78:2497–2504. [PubMed: 20308301]

Stricklett PK, Hughes AK, Kohan DE. Inhibition of p38 mitogen-activated protein kinase ameliorates cytokine up-regulated shigatoxin-1 toxicity in human brain microvascular endothelial cells. J Infect Dis. 2005; 191:461–471. [PubMed: 15633106]

Sugatani J, Komiyama N, Mochizuki T, Hoshino M, Miyamoto D, Igarashi T, Hoshi S, Miwa M. Urinary concentrating defect in rats given Shiga toxin: elevation in urinary AQP2 level associated with polyuria. Life Sci. 2002; 71:171–189. [PubMed: 12031687]

Szabady RL, Lokuta MA, Walters KB, Huttenlocher A, Welch RA. Modulation of neutrophil function by a secreted mucinase of *Escherichia coli* O157:H7. PLoS Pathog. 2009; 5:e1000320. [PubMed: 19247439]

Takeuchi O, Akira S. Toll-like receptors; their physiological role and signal transduction system. Int Immunopharmacol. 2001; 1:625–635. [PubMed: 11357875]

Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet. 2005; 365:1073–1086. [PubMed: 15781103]

Taylor CM, Milford DV, Rose PE, Roy TC, Rowe B. The expression of blood group P1 in post-enteropathic haemolytic uraemic syndrome. Pediatr Nephrol. 1990; 4:59–61. [PubMed: 2206883]

Taylor FB Jr, Tesh VL, DeBault L, Li A, Chang AC, Kosanke SD, Pysher TJ, Siegler RL. Characterization of the baboon responses to Shiga-like toxin: descriptive study of a new primate model of toxic responses to Stx-1. Am J Pathol. 1999; 154:1285–1299. [PubMed: 10233866]

Tazzari PL, Ricci F, Carnicelli D, Caprioli A, Tozzi AE, Rizzoni G, Conte R, Brigotti M. Flow cytometry detection of shiga toxins in the blood from children with hemolytic uremic syndrome. Cytom B Clin Cytom. 2004; 61:40–44.

Te Loo DM, Monnens LA, van Der Velden TJ, Vermeer MA, Preyers F, Demacker PN, van Den Heuvel LP, van Hinsbergh VW. Binding and transfer of verocytotoxin by polymorphonuclear leukocytes in hemolytic uremic syndrome. Blood. 2000; 95:3396–3402. [PubMed: 10828021]

Te Loo DM, van Hinsbergh VW, van den Heuvel LP, Monnens LA. Detection of verocytotoxin bound to circulating polymorphonuclear leukocytes of patients with hemolytic uremic syndrome. J Am Soc Nephrol. 2001; 12:800–806. [PubMed: 11274241]

Te Loo DM, Monnens L, van der Velden T, Karmali M, van den Heuvel L, van Hinsbergh V. Shiga toxin-1 affects nitric oxide production by human glomerular endothelial and mesangial cells. Pediatr Nephrol. 2006; 21:1815–1823. [PubMed: 16944213]

Tesh VL, Samuel JE, Perera LP, Sharefkin JB, O'Brien AD. Evaluation of the role of Shiga and Shiga-like toxins in mediating direct damage to human vascular endothelial cells. J Infect Dis. 1991; 164:344–352. [PubMed: 1649877]

Tesh VL, Burris JA, Owens JW, Gordon VM, Wadolkowski EA, O'Brien AD, Samuel JE. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect Immun. 1993; 61:3392–3402. [PubMed: 8335369]

Thorner PS, Ho M, Eremina V, Sado Y, Quaggin S. Podocytes contribute to the formation of glomerular crescents. J Am Soc Nephrol. 2008; 19:495–502. [PubMed: 18199804]

Thurman JM, Marians R, Emlen W, Wood S, Smith C, Akana H, Holers VM, Lesser M, Kline M, Hoffman C, Christen E, Trachtman H. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2009; 4:1920–1924. [PubMed: 19820137]

Trachtman H, Christen E, Cnaan A, Patrick J, Mai V, Mishra J, Jain A, Bullington N, Devarajan P. Urinary neutrophil gelatinase-associated lipocalcin in D+HUS: a novel marker of renal injury. Pediatr Nephrol. 2006; 21:989–994. [PubMed: 16773412]

Tryggvason K, Patrakka J, Wartiovaara J. Hereditary proteinuria syndromes and mechanisms of proteinuria. N Engl J Med. 2006; 354:1387–1401. [PubMed: 16571882]

Tsai HM, Chandler WL, Sarode R, Hoffman R, Jelacic S, Habeeb RL, Watkins SL, Wong CS, Williams GD, Tarr PI. von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. Pediatr Res. 2001; 49:653–659. [PubMed: 11328948]

Turi S, Nemeth I, Vargha I, Matkovics B. Oxidative damage of red blood cells in haemolytic uraemic syndrome. Pediatr Nephrol. 1994; 8:26–29. [PubMed: 8142220]

Tzipori S, Sheoran A, Akiyoshi D, Donohue-Rolfe A, Trachtman H. Antibody therapy in the management of shiga toxin-induced hemolytic uremic syndrome. Clin Microbiol Rev. 2004; 17:926–941. Table of contents. [PubMed: 15489355]

Uchida H, Kiyokawa N, Horie H, Fujimoto J, Takeda T. The detection of Shiga toxins in the kidney of a patient with hemolytic uremic syndrome. Pediatr Res. 1999; 45:133–137. [PubMed: 9890621]

van de Kar NC, Monnens LA, Karmali MA, van Hinsbergh VW. Tumor necrosis factor and interleukin-1 induce expression of the verocytotoxin receptor globotriaosylceramide on human endothelial cells: implications for the pathogenesis of the hemolytic uremic syndrome. Blood. 1992; 80:2755–2764. [PubMed: 1333300]

van de Kar NC, Monnens LA, Van Hinsbergh VW. Tumor necrosis factor and interleukin 1 induce expression of the glycolipid verotoxin receptor in human endothelial cells. Implications for the pathogenesis of the haemolytic uraemic syndrome. Behring Inst Mitt. 1993; 92:202–209. [PubMed: 8250811]

van de Kar NC, van Hinsbergh VW, Brommer EJ, Monnens LA. The fibrinolytic system in the hemolytic uremic syndrome: in vivo and in vitro studies. Pediatr Res. 1994; 36:257–264. [PubMed: 7970942]

Van Geet C, Proesmans W, Arnout J, Vermylen J, Declerck PJ. Activation of both coagulation and fibrinolysis in childhood hemolytic uremic syndrome. Kidney Int. 1998; 54:1324–1330. [PubMed: 9767551]

van Setten PA, Monnens LA, Verstraten RG, van den Heuvel LP, van Hinsbergh VW. Effects of verocytotoxin-1 on nonadherent human monocytes: binding characteristics, protein synthesis, and induction of cytokine release. Blood. 1996; 88:174–183. [PubMed: 8704172]

van Setten PA, van Hinsbergh VW, Van den Heuvel LP, van der Velden TJ, van de Kar NC, Krebbers RJ, Karmali MA, Monnens LA. Verocytotoxin inhibits mitogenesis and protein synthesis in purified human glomerular mesangial cells without affecting cell viability: evidence for two distinct mechanisms. J Am Soc Nephrol. 1997a; 8:1877–1888. [PubMed: 9402090]

van Setten PA, van Hinsbergh VW, van der Velden TJ, van de Kar NC, Vermeer M, Mahan JD, Assmann KJ, van den Heuvel LP, Monnens LA. Effects of TNF alpha on verocytotoxin cytotoxicity in purified human glomerular microvascular endothelial cells. Kidney Int. 1997b; 51:1245–1256. [PubMed: 9083293]

van Setten PA, van Hinsbergh VW, van den Heuvel LP, Preyers F, Dijkman HB, Assmann KJ, van der Velden TJ, Monnens LA. Monocyte chemoattractant protein-1 and interleukin-8 levels in urine and serum of patents with hemolytic uremic syndrome. Pediatr Res. 1998; 43:759–767. [PubMed: 9621985]

Vandewalle A. Toll-like receptors and renal bacterial infections. Chang Gung Med J. 2008; 31:525–537. [PubMed: 19241891]

Vaughan MR, Quaggin SE. How do mesangial and endothelial cells form the glomerular tuft? J Am Soc Nephrol. 2008; 19:24–33. [PubMed: 18178797]

Viau A, El Karoui K, Laouari D, Burtin M, Nguyen C, Mori K, Pillebout E, Berger T, Mak TW, Knebelmann B, Friedlander G, Barasch J, Terzi F. Lipocalin 2 is essential for chronic kidney disease progression in mice and humans. J Clin Invest. 2010; 120:4065–4076. [PubMed: 20921623]

Viisoreanu D, Polanowska-Grabowska R, Suttitanamongkol S, Obrig TG, Gear AR. Human platelet aggregation is not altered by Shiga toxins 1 or 2. Thromb Res. 2000; 98:403–410. [PubMed: 10828480]

Wachowicz B, Saluk J, Kaca W. Response of blood platelets to Proteus mirabilis lipopolysaccharide. Microbiol Immunol. 1998; 42:47–49. [PubMed: 9525779]

Waddell T, Cohen A, Lingwood CA. Induction of verotoxin sensitivity in receptor-deficient cell lines using the receptor glycolipid globotriosylceramide. Proc Natl Acad Sci U S A. 1990; 87:7898–7901. [PubMed: 2236008]

Wahome PG, Bai Y, Neal LM, Robertus JD, Mantis NJ. Identification of small-molecule inhibitors of ricin and shiga toxin using a cell-based high-throughput screen. Toxicon. 2010; 56:526–534. [PubMed: 20493201]

Warnier M, Romer W, Geelen J, Lesieur J, Amessou M, van den Heuvel L, Monnens L, Johannes L. Trafficking of Shiga toxin/Shiga-like toxin-1 in human glomerular microvascular endothelial cells and human mesangial cells. Kidney Int. 2006; 70:2085–2092. [PubMed: 17063173]

Watanabe-Takahashi M, Sato T, Dohi T, Noguchi N, Kano F, Murata M, Hamabata T, Natori Y, Nishikawa K. An orally applicable Shiga toxin neutralizer functions in the intestine to inhibit the intracellular transport of the toxin. Infect Immun. 2010; 78:177–183. [PubMed: 19858299]

Williams JM, Boyd B, Nutikka A, Lingwood CA, Barnett Foster DE, Milford DV, Taylor CM. A comparison of the effects of verocytotoxin-1 on primary human renal cell cultures. Toxicol Lett. 1999; 105:47–57. [PubMed: 10092056]

Wilson C, Foster GH, Bitzan M. Silencing of Bak ameliorates apoptosis of human proximal tubular epithelial cells by Escherichia coli-derived Shiga toxin 2. Infection. 2005; 33:362–367. [PubMed: 16258868]

Winter KR, Stoffregen WC, Dean-Nystrom EA. Shiga toxin binding to isolated porcine tissues and peripheral blood leukocytes. Infect Immun. 2004; 72:6680–6684. [PubMed: 15501802]

Wolski VM, Soltyk AM, Brunton JL. Tumour necrosis factor alpha is not an essential component of verotoxin 1-induced toxicity in mice. Microb Pathog. 2002; 32:263–271. [PubMed: 12137753]

Wu X, He Y, Jing Y, Li K, Zhang J. Albumin overload induces apoptosis in renal tubular epithelial cells through a CHOP-dependent pathway. Omics. 2010; 14:61–73. [PubMed: 20141329]

Yamamoto M, Akira S. Lipid A receptor TLR4-Mediated signaling pathways. Adv Exp Med Biol. 2010; 667:59–68. [PubMed: 20665200]

Yannariello-Brown J, Wewer U, Liotta L, Madri JA. Distribution of a 69-kD laminin-binding protein in aortic and microvascular endothelial cells: modulation during cell attachment, spreading, and migration. J Cell Biol. 1988; 106:1773–1786. [PubMed: 2967300]

Zanchi C, Zoja C, Morigi M, Valsecchi F, Liu XY, Rottoli D, Locatelli M, Buelli S, Pezzotta A, Mapelli P, Geelen J, Remuzzi G, Hawiger J. Fractalkine and CX3CR1 mediate leukocyte capture by endothelium in response to Shiga toxin. J Immunol. 2008; 181:1460–1469. [PubMed: 18606701]

Zipfel PF, Heinen S, Skerka C. Thrombotic microangiopathies: new insights and new challenges. Curr Opin Nephrol Hypertens. 2010; 19:372–378. [PubMed: 20539230]

Zoja C, Morigi M, Remuzzi G. The role of the endothelium in hemolytic uremic syndrome. J Nephrol. 2001; 14(4):S58–S62. [PubMed: 11798147]

Zoja C, Angioletti S, Donadelli R, Zanchi C, Tomasoni S, Binda E, Imberti B, te Loo M, Monnens L, Remuzzi G, Morigi M. Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. Kidney Int. 2002; 62:846–856. [PubMed: 12164866]

Zoja C, Morigi M, Remuzzi G. Proteinuria and phenotypic change of proximal tubular cells. J Am Soc Nephrol. 2003; 14(1):S36–S41. [PubMed: 12761237]

Zoja C, Buelli S, Morigi M. Shiga toxin-associated hemolytic uremic syndrome: pathophysiology of endothelial dysfunction. Pediatr Nephrol. 2010; 25:2231–2240. [PubMed: 20424866]