Hemolytic Uremic Syndrome

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Hemolytic uremic syndrome (HUS) is a disease of nonimmune (Coombs negative) hemolytic anemia, low platelet count, and renal impairment (1). Anemia is severe and microangiopathic in nature, with fragmented red blood cells (schistocytes) in the peripheral smear, high serum lactate dehydrogenase (LDH), circulating free hemoglobin, and reticulocytes. Platelet count is $<60{,}000/mm^3$ in most cases (1).

In children, the disease is most commonly triggered by Shiga-like toxin (Stx)-producing *Escherichia coli* (Stx-*E. coli*) and manifests with diarrhea (D^+HUS), often bloody. Cases of Stx-*E. coli* HUS—approximately 25% (2)—which, however do not present with diarrhea, have also been reported (3). Acute renal failure manifests in 55 to 70% of cases (4–6); however, renal function recovers in most of them (up to 70% in various series) (1,3,6,7).

Non–Shiga toxin-associated HUS (non–Stx-HUS) comprises a heterogeneous group of patients in whom an infection by Stx-producing bacteria could be excluded as cause of the disease. It can be sporadic or familial (*i.e.*, more than one member of a family affected by the disease and exposure to Stx-*E. coli* excluded). Collectively, non–Stx-HUS forms have a poor outcome. Up to 50% of cases progress to ESRD or have irreversible brain damage, and 25% may die during the acute phase of the disease (8–10). Genetic studies have recently documented that the familial form is associated with genetic abnormalities of complement regulatory proteins, and evidence is now emerging that similar genetic alterations can predispose to sporadic cases of non–Stx-HUS as well. Major recent advances in the field of Stx-HUS and non–Stx-HUS are summarized in Table 1.

Microvascular lesion of HUS consists of vessel wall thickening with endothelial swelling and accumulation of proteins and cell debris in the subendothelial layer, creating a space between endothelial cells and the underlying basement membrane of affected microvessels (1,3). In Stx-HUS, the lesion is mainly confined to the glomerular tuft and is noted in an early phase of the disease. Examination of biopsies taken several months after the disease onset showed that most glomeruli are normal, whereas 15 to 20% eventually became sclerotic (11,12). Arterial thrombosis does occur but is uncommon and seems to be a proximal extension of the glomerular lesion (11,12).

Stx-Associated HUS

Epidemiology

In 70% of cases in North America and Western Europe, Stx-HUS is secondary to infection with the *E. coli* serotype O157:H7 (13–19). This serotype has a unique biochemical property (lack of sorbitol fermentation) as to render it readily distinguishable from other fecal *E. coli* (20). However, many other *E. coli* serotypes (O111:H8, O103:H2, O121, O145, O26, and O113 [13,16,21–23]) have been shown to cause Stx-HUS. Infection by Stx-producing *Shigella dysenteriae* serotype 1 has been commonly linked to Stx-HUS in developing countries of Asia (24) and Africa (25) but rarely in industrialized countries (26).

After exposure to Stx-*E. coli*, 38 to 61% of individuals develop hemorrhagic colitis and 3 to 9% (in sporadic infections) to 20% (in epidemic forms) progress to overt HUS (5,27). The overall incidence of Stx-HUS is estimated to be 2.1 cases per 100,000 persons/yr, with a peak incidence in children who are younger than 5 yr (6.1 per 100,000/yr), and the lowest rate in adults who are 50 to 59 yr of age (0.5 per 100,000/yr) (1). The incidence of the disease parallels the seasonal fluctuation of *E. coli* O157:H7 infections with a peak in warmer months, between June and September. In the United States, approximately 70,000 illnesses and 60 deaths have been attributed annually to Stx-HUS (28). In Argentina and Uruguay, *E. coli* infections are endemic and Stx-HUS is a common cause of acute renal failure in children (23,29,30), with an estimated incidence rate of 10.5 per 100,000/yr (31). An association between traditional extensive production of cattle with endemic HUS in Argentina has been proposed, as supported by detection of Stx-producing *E. coli* strains—mainly O8, O25, O103, O112, O113, O145, O171, and O174 serotypes—in stool samples from 39% of Argentine healthy young beef steers (31).

Stx-producing *E. coli* colonize healthy cattle intestine but also have been isolated from deer, sheep, goats, horses, dogs, birds, and flies (1,32). They are found in manure and water troughs in farms, which explains the increased risk for infection in people who live in rural areas. Humans become infected from contaminated milk, meat, and water—water-borne outbreaks have occurred as a result of drinking and swimming in unchlorinated water (21)—or from contact with infected animals, humans, or either's excreta (27,33,34) and occasionally through

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Table 1. HUS: Major advances in recent years[a]

Stx-HUS	
1994–2004	Description of the crystal structure of Stx-1 and Stx-2 (46,52)
1993–2001	Specific Stx surface receptors (globotriaosylceramide, Gb3) were identified on human endothelial cells, platelets, monocytes, erythrocytes, and polymorphonuclear cells (57–66)
1995–2003	Identification of the molecular mechanisms by which Stx promotes leukocyte adhesion to endothelial cells and induces thrombus formation (71–74)
2004	Description of the beneficial effect of angiotensin-converting enzyme inhibitors on long-term renal outcome in children with renal sequelae after severe Stx-HUS (83,84)
2002–2003	Reports on the good outcome of kidney transplantation in children with Stx-HUS (85–87)
Non–Stx-HUS	
1999	Description of high incidence of hypocomplementemia (low C3 levels) in familial forms of non-Stx-HUS (98)
1998	Linkage mapping of familial HUS on human chromosome 1q32 containing the regulator of complement activation gene cluster (110)
1998–2004	Identification of 50 mutations in factor H gene in familial and sporadic non–Stx-HUS (99–102,110,114,119–121)
2002–2004	Localization (in SCR19–20) of the domain responsible for inactivation of surface-bound C3b by factor H (115–118)
2002–2004	Demonstration that mutations found in patients with non–Stx-HUS cause loss of the capability of factor H to bind polyanions on endothelial cells and extracellular matrix and to bind C3b (117,118,123)
2003	Mutations in another complement regulatory gene, MCP, in non–Stx-HUS (126,127)
1997–2003	Description of high incidence of recurrence on the kidney graft in patients with non–Stx-HUS (1,85,86,99–101,153,154)
2003–2004	Complement inhibitors are being clinically available (163–167)

[a]HUS, hemolytic uremic syndrome; Stx, Shiga toxin; MCP, monocyte chemoattractant protein.

environmental contamination (17). Meat is contaminated at slaughter. Internalization of the microorganism during grinding renders it capable of surviving cooking (27). Fruits and vegetables may also be contaminated, including radish sprouts, lettuce, and apple cider. Unpasteurized apple juice has been implicated in several outbreaks (35). Person-to-person transmission has been reported in child care and long-term care facilities (27).

Clinical Phenotype

The disease is characterized by prodromal diarrhea followed by acute renal failure. The average interval between *E. coli* exposure and illness is 3 d (range, 1 to 8). Illness typically begins with abdominal cramps and nonbloody diarrhea; diarrhea may become hemorrhagic in 70% of cases usually within 1 or 2 d (36). Vomiting occurs in 30 to 60% of cases, and fever occurs in 30%. Leukocyte count is usually elevated, and a barium enema may demonstrate "thumb-printing," suggestive of edema and submucosal hemorrhage, especially in the region of the ascending and transverse colon. HUS is usually diagnosed 6 d after the onset of diarrhea (1). After infection, Stx-*E. coli* may be shed in the stools for several weeks after the symptoms are resolved, particularly in children <5 yr of age (1). Diagnosis rests on detection of Stx-*E. coli* in stool cultures. Serologic tests for antibodies to Stx and O157 LPS can be done in research laboratories, and tests are being developed for rapid detection of *E. coli* O157:H7 and Stx in stools. Bloody diarrhea, fever, vomiting, elevated leukocyte count, extremes of age, and female gender as well as the use of antimotility agents (37) have been associated with an increased risk of HUS after *E. coli* infection (27).

Stx-HUS is not a benign disease. Seventy-percent of patients who develop HUS require red blood cell transfusions, 50% need dialysis, and 25% have neurologic involvement, including stroke, seizure, and coma (6,27,38). Although mortality for infants and young children in industrialized countries decreased when dialysis became available, as well as after the introduction of intensive care facilities, still 3 to 5% of patients die during the acute phase of Stx-HUS (6). A recent meta-analysis of 49 published studies (3476 patients, mean follow-up of 4.4 yr) describing long-term prognosis of patients who survived an episode of Stx-HUS reported death or permanent ESRD in 12% of patients and GFR <80 ml/min per 1.73 m^2 in 25% (38). The severity of acute illness, particularly central nervous system symptoms, and the need for initial dialysis were strongly associated with a worse long-term prognosis (4,38). Stx-HUS that is precipitated by *S. dysenteriae* infection is almost invariably complicated by bacteremia and septic shock, systemic intravascular coagulation, and acute cortical necrosis and renal death and has a high mortality rate (approximately 30%) (39).

History of a Discovery

E. coli has been associated with hemorrhagic colitis and organ failure, including kidney failure. In 1927, Albert Adam first reported an epidemia of bloody diarrhea of infants caused by a special type of Bacterium *coli*. Such bacterium was biochemically unique in that fermentation properties were different from known *E. coli* strains (40). In 1947, the *E. coli* O111:B4 was found in the stools of >90% of infants with epidemic diarrhea but never in their blood (41). A filterable agent—we now know that this was likely Stx—that caused diarrhea in calves and was lethal to mice was isolated from the stools of these children. A few years later, it was found that most severe cases of O111: B4-induced epidemic diarrhea were associated with purpura, anuria, and neurologic signs. Autopsy material revealed thrombosis of capillary and precapillary arterioles in lungs, liver, brain, and kidneys, as well as glomerular tuft occlusion by fibrin thrombi (42). These early findings were taken to indicate that a toxin, possibly released by the *E. coli*, induced hemorrhagic necrosis of the gastrointestinal mucosa and—once absorbed into the blood stream—caused microvascular thrombosis of kidneys and the other organs. Several years later, in 1977, Konowalciuck *et al.* (43) noted that *E. coli* that was isolated from patients with diarrhea produced a toxin similar to the one of *S. dysenteriae type 1* (Stx) found cytopathic to Vero cells (African green monkey kidney cells). Karmali *et al.* (14) found an increased Stx activity in fecal filtrates and increased Stx-neutralizing antibody titer in sera from children who had *E-coli* O157:H7 infection an had received a diagnosis of HUS.

Shiga Toxin or Shiga Toxins?

The Stx associated with *E. coli* are designated by a number. Stx-1 is almost identical to Stx from *S. dysenteriae type 1*, differing by a single amino acid, and is 50% homologous with Stx-2 (44–46). Despite their similar sequences, Stx-1 and Stx-2 cause different degrees and types of tissue damage as documented by the higher pathogenicity of strains of *E. coli* that produce only Stx-2 than of those that produce Stx-1 alone (47–49). In a recent study in children who become infected by Stx-*E. coli*, *E. coli* strains that produced Stx-2 were most commonly associated with HUS, whereas most strains that were isolated from children who had diarrhea alone or remained asymptomatic produced only Stx-1 (50). This is also true in mice and baboons (45,51).

Both Stx-1 and Stx-2 are 70-kD AB5 holotoxins that are composed of a single A subunit of 32-kD and five 7.7-kD B subunits (52) (Figure 1). It is interesting that a new AB5 toxin that comprises a single 35-kD A subunit and a pentamer of 13-kD B subunits has been recently isolated from a highly virulent *E. coli* strain (0113:H21) that was responsible for an outbreak of HUS (53), which may represent the prototype of a new class of toxins, accounting for HUS associated with strains of *E. coli* that do not produce Stx.

After oral ingestion, Stx-*E. coli* reaches the gut and closely adheres to the epithelial cells of the gastrointestinal mucosa through a 97-kD outer membrane protein, intimin (54). Stx then are picked up by polarized gastrointestinal cells via transcellular pathways (55) and translocate into the circulation, probably

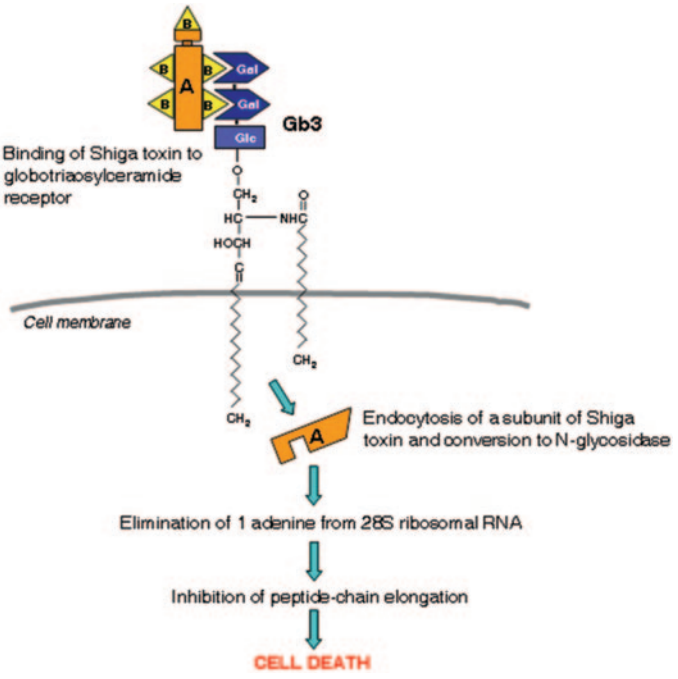

Figure 1. Binding and mechanism of action of Shiga-like toxin. The B subunits of Shiga toxin (Stx) molecules attach to galactose (gal) disaccharides of globotriaosylceramide (Gb3) receptors on the membrane of monocytes, polymorphonuclear cells, platelets, glomerular endothelial cells, and tubular epithelial cells. The toxin is internalized via retrograde transport through the Golgi complex. Then the A and B subunits dissociate, and the A subunit is translocated to the cytosol. The A subunit blocks peptide chain elongation by eliminating one adenine from the 28S ribosomal RNA.

facilitated by the transmigration of neutrophils (PMN) (56), which increase paracellular permeability. The route of transport of Stx from the intestine to the kidney has been greatly debated. *In vitro* experiments have shown that Stx can bind to human erythrocytes (57), platelets (58), and activated monocytes (59). However, more recent studies have underpinned a role for PMN in Stx transfer in the blood because Stx rapidly and completely bind to PMN when incubated with human blood (60). Consistently, Stx bound to circulating PMN have been detected in the blood of patients with Stx-HUS (61). The Stx receptor on PMN has a 100-fold lower affinity than the high-affinity receptor expressed on glomerular endothelial cells. *In vitro*, in co-cultures, PMN loaded with Stx transfer the ligand to glomerular endothelial cells so that at the end of the incubation, Stx molecules were found on glomerular endothelial cells but no more on PMN (60).

Binding of Stx to target cells is dependent on B subunits and occurs via the terminal digalactose moiety of the glycolipid cell surface receptor globotriaosylceramide Gb3 (Figure 1). Stx-1 and Stx-2 bind to different epitopes on the Gb3 molecule, and they also differ in binding affinity and kinetics (62). Surface plasmon resonance analysis showed that Stx-1 easily binds to and detaches from Gb3, in contrast to Stx-2, which binds slowly but also dissociates very slowly, thus staying on the cells long

enough to be incorporated (62). The latter could explain why Stx-2 is 1000-fold more toxic than Stx-1 on human endothelial cells *in vitro* (63).

Cultured human microvascular endothelial cells are more susceptible to the toxic effects of Stx than large-vessel endothelium (64). This is consistent with data that the number of Gb3 receptors expressed on human microvascular endothelial cells is 50-fold higher than in endothelial cells from human umbilical veins (65). In human glomerular endothelial cells, Gb3 expression and Stx toxicity are further increased upon exposure to TNF-α (66), in turn released by monocytes in response to Stx binding (59). Altogether these data provide the biochemical basis for the preferential localization of microangiopathic lesions to renal vasculature in HUS in humans.

After internalization by receptor-mediated endocytosis, Stx are carried by retrograde transport through the Golgi complex to the endoplasmic reticulum, where the A and B subunits likely dissociate (Figure 1). Then the A subunit is translocated to the cytosol and nuclear envelope, where it enzymatically blocks protein synthesis (67) (Figure 1). Stx-1 and Stx-2 also induce endothelial apoptosis (68,69) possibly by inhibiting the expression of the antiapoptotic Bcl-2 family member, Mcl-1 (70).

For many years, it was assumed that the only relevant biologic activity of Stx was the block of protein synthesis and destruction of endothelial cells. Recently, however, it has been shown that treatment of endothelial cells with sublethal doses of Stx, exerting minimal influence on protein synthesis, leads to increased mRNA levels and protein expression of chemokines, such as IL-8 and monocyte chemoattractant protein-1 (MCP-1) and cell adhesion molecules, a process preceded by NF-κB activation (71). Analysis of genome-wide expression pattern of human endothelial cells stimulated with sublethal doses of Stx evidenced 25 and 24 genes upregulated by Stx-1 and Stx-2, respectively, mostly encoding for chemokines and cytokines, cell adhesion molecules, including P-selectin and ICAM-1, and transcription factors (EGR-1, NF-κB2, and NF-κBIA) (72). Chemokines and cytokines are likely involved in the chemoattraction and activation of neutrophils. Adhesion molecules seem to play a critical role in mediating binding of inflammatory cells to the endothelium. This is supported by adhesion experiments under flow showing that Stx-2 treatment enhanced the number of leukocytes that adhere and migrate across a monolayer of human endothelial cells (73). Preventing IL-8 and MCP-1 overexpression by adenovirus-mediated blocking of NF-κB inhibited the adhesion and transmigration of leukocytes (71).

Taken together, these findings indicate that Stx, by altering endothelial cell adhesion properties and metabolism, favor leukocyte-dependent inflammation. The latter activates endothelial cells that lose thromboresistance, which ultimately leads to microvascular thrombosis. Evidence for such sequence of events has been obtained in experiments of whole blood flowing on human microvascular endothelial cells, pre-exposed to Stx-1, at high shear stress (74). Finding that in such circumstances early platelet activation and adhesion takes place, followed by the formation of organized thrombi dependent on endothelial P-selectin and PECAM-1, offers a plausible pathophysiologic pathway for microvascular thrombosis in HUS. The above report could also be taken as a demonstration of a link between bacteria and their products and arterial thrombosis, as suggested in the accompanying commentary (75).

In vivo evidence of coagulation disturbances, *i.e.*, increase in prothrombin fragment 1 + 2, has been found (36) in children who developed HUS upon *E. coli* O157:H7 infection. Although early studies suggested that fibrinolysis is augmented in Stx-HUS (76), more recent work revealed the presence of higher-than-normal levels of plasminogen-activator inhibitor type 1, indicating that fibrinolysis is substantially inhibited (36).

Is There Any Effective Treatment for Stx-HUS?

There is no treatment of proven value, and care during the acute phase of the illness is still merely supportive with no substantial changes as compared with the past (Table 2). There is no clear consensus on whether antibiotics should be admin-

Table 2. Classification and treatment of different forms of HUS[a]

Disease	Causes	Treatment
Stx-HUS	Stx-producing *Escherichia coli*	Supportive
	Shigella dysenteriae type 1	Supportive, antibiotics
Non–Stx-HUS	Bacteria (*Streptococcus pneumoniae*)	Antibiotics, no plasma
sporadic	Viruses (HIV)	Plasma
	Drugs (antineoplastic, antiplatelet, immunosuppressive)	Drug withdrawal, plasma
	Pregnancy associated	Delivery, plasma
	Postpartum	Plasma
	Systemic diseases	
	lupus	Steroids, plasma
	scleroderma	BP control
	antiphospholipid syndrome	Oral anticoagulants
	Idiopathic	Plasma
	Genetic (factor H, MCP, factor I)	Plasma
familial	Genetic (factor H, MCP, factor I), plasma	Plasma

istered to treat Stx-*E. coli* infection. Wong *et al.* (77) showed that antibiotic therapy at the stage of gastrointestinal infection with Stx-*E. coli* increases—by approximately 17-fold—the risk for full-blown HUS. It was postulated that antibiotic-induced injury to the bacterial membrane might favor the acute release of large amounts of toxins. However, a recent meta-analysis on 26 reports failed to show a higher risk for HUS associated with antibiotic administration (78). Of note, in the study by Wong *et al.*, none of the patients had bacteremia. Although bacteremia is very common in Stx-HUS precipitated by *S. dysenteriae type 1* and these patients eventually progress to death unless antibiotics are started early enough (79,80), such complication is only exceptionally found in Stx-HUS sustained by *E. coli* O157:H7 infection. However, a recent report of an adult patient with *E. coli* O157:H7–induced HUS with bacteremia and urinary tract infection showed that early antibiotic therapy rapidly resolved hematologic and renal abnormalities (81). On the basis of available data, we suggest that in patients with Stx-*E. coli* gastrointestinal infection, antibiotics should be avoided unless in cases with sepsis.

A study with an Stx-binding agent, SYNSORB Pk, composed of particles of silicon linked to the globotriaosylceramide, given orally (82), failed to find any effect of SYNSORB over placebo. Most treatments, including plasma therapy, intravenous IgG, fibrinolytic agents, antiplatelet drugs, corticosteroids, and antioxidants (38), have been shown to be ineffective in controlled clinical trials in the acute phase of the disease (38). Careful BP control and renin-angiotensin system blockade may be particularly beneficial on the long term for patients who experience chronic renal disease after an episode of Stx-HUS. A recent study in 45 children who had renal sequelae of HUS and were followed for 9 to 11 yr documented that early restriction of proteins and use of angiotensin-converting enzyme inhibitors may have a beneficial effect on long-term renal outcome, as documented by a positive slope of 1/Cr values over time in treated patients (83). In another study, 8 to 15 yr of treatment with angiotensin-converting enzyme inhibitors after severe Stx-HUS normalized BP, reduced proteinuria, and improved GFR (84).

Finally, kidney transplant should be considered as an effective and safe treatment for children who progress to ESRD. Indeed, the outcome of renal transplantation is good in children with Stx-HUS: Recurrence rates range from 0 to 10% (85,86), and graft survival at 10 yr is even better than in control children who had other diseases and received a transplant (87).

Non–Stx-HUS

Epidemiology and Clinical Features

Non–Stx-HUS is less common than Stx-HUS and accounts for only 5 to 10% of all cases of the disease (1,88). It may manifest at all ages but is more frequent in adults. According to a recent U.S. study, the incidence of non–Stx-HUS in children is approximately one tenth that of Stx-HUS (10), corresponding to approximately 2 cases/yr per 1000,000 total population. At variance with Stx-HUS, there is no clear causative agent or seasonal pattern. The onset may be preceded by features of the nephrotic syndrome. A diarrhea prodrome is rarely observed (D^-HUS) (1,3,10,89). Non–Stx-HUS can occur sporadically or in families.

Sporadic Non–Stx-HUS. A wide variety of triggers for sporadic non–Stx-HUS have been identified, including various nonenteric infections, viruses, drugs, malignancies, transplantation, pregnancy, and other underlying medical conditions (scleroderma, antiphospholipid syndrome, lupus; Table 2). Infection caused by *Streptococcus pneumoniae* accounts for 40% of non–Stx-HUS and 4.7% of all causes of HUS in children in the United States (10). Neuroaminidase produced by *S. pneumoniae*, by removing sialic acids from the cell membranes, exposes Thomsen-Friedenreich antigen to preformed circulating IgM antibodies, which bind to this neoantigen on platelet and endothelial cells and cause platelet aggregation and endothelial damage (90,91). The clinical picture is usually severe, with respiratory distress, neurologic involvement, and coma and a mortality rate of 50% (91).

Categories of drugs that have been most frequently reported to induce non–Stx-HUS include anticancer molecules (mitomycin, cisplatin, bleomycin, and gemcitabine), immunotherapeutic (cyclosporine, tacrolimus, OKT3, IFN, and quinidine), and antiplatelet (ticlopidine and clopidogrel) agents (92). The risk for developing HUS after mitomycin is 2 to 10%. The onset is delayed, occurring almost 1 yr after starting treatment. The prognosis is poor, with up to 75% mortality at 4 mo (92).

Posttransplantation HUS is being reported with increasing frequency (1,93). It may ensue for the first time in patients who never experienced the disease (*de novo* posttransplantation HUS) or may affect patients whose primary cause of ESRD was HUS (recurrent posttransplantation HUS, discussed later in this review). *De novo* posttransplantation HUS might occur in patients who receive renal transplants and other organs, as a consequence of the use of calcineurin inhibitors or of humoral (C4b positive) rejection. It occurs in 5 to 15% of renal transplant patients who receive cyclosporine and in approximately 1% of those who are given tacrolimus (94).

Pregnancy-associated HUS may occasionally develop as a complication of preeclampsia. Some patients progress to a life-threatening variant of preeclampsia with severe thrombocytopenia, microangiopathic hemolytic anemia, renal failure, and liver involvement (HELLP syndrome). These forms are always an indication for prompt delivery that is usually followed by complete remission (95). Postpartum HUS manifests within 3 mo of delivery in most cases. The outcome is usually poor, with 50 to 60% mortality; residual renal dysfunction and hypertension are the rule in surviving patients (96). Of note, in approximately 50% of cases of sporadic non–Stx-HUS, no clear triggering conditions could be found (idiopathic HUS) (1).

Familial Non–Stx-HUS. Familial forms account for fewer than 3% of all cases of HUS. Both autosomal dominant and autosomal recessive forms of inheritance have been noted (97). In autosomal recessive HUS, the onset is usually early in childhood. The prognosis is poor, with a mortality rate of 60 to 70%. Recurrences are very frequent. Autosomal dominant HUS has an adult onset in most cases; the prognosis is poor, with a cumulative incidence of death or ESRD of 50 (98) to 90% (97).

Recent studies have documented that familial HUS may be

caused by genetic abnormalities of proteins involved in the regulation of the complement system. Similar genetic abnormalities have been found in sporadic non–Stx-HUS, mainly in idiopathic forms (99,100) but also in rare cases of pregnancy-associated (99) and postpartum HUS (three patients) (101,102), ticlopidine-induced HUS (one patient) (99), and postinfectious HUS (*Neisseria meningitidis*; one patient) (103).

Genetic Studies

Reduced serum levels of the third component (C3) of complement have been reported since 1974 in both familial and sporadic forms of non–Stx-HUS (98,104,105). Low C3 levels likely reflect C3 consumption in the microvasculature rather than defective synthesis, as documented by granular C3 deposits in glomeruli and arterioles of HUS patients (106,107) and by increased C3 breakdown products in sera. By contrast, levels of the fourth fraction of complement, C4, are usually normal (98). Persistent and remarkably depressed C3 levels found in patients with familial HUS, even in the unaffected relatives (98), suggested an inherited defect causing hyperactivation of the complement cascade.

The complement system consists of several plasma- and membrane-associated proteins that are organized in three activation pathways: The classical, the lectin, and the alternative pathway (108,109) (Figure 2). Upon activation by molecules on the surface of microorganisms, these pathways result in the formation of protease complexes, the C3 convertases, which cleave C3 generating C3b. The classic/lectin convertases are formed by C2 and C4 fragments, whereas the generation of the alternative pathway convertase requires the cleavage of C3 but not of C4. Thus, low C3 levels in patients with HUS in the presence of normal C4 indicate a selective activation of the alternative pathway (98).

Upon generation, C3b deposits on bacterial surfaces, which leads to opsonization for phagocytosis by PMN and macrophages. C3b also participates to the formation of the C5 convertases that cleaves C5 and initiates assembly of the membrane attack complex that causes cell lysis. The human complement system is highly regulated as to prevent nonspecific damage to host cells and limit deposition of C3b to the surface of pathogens. This fine regulation is based on a number of membrane-anchored (CR1, DAF, MCP, and CD59) and fluid-phase (factor H) regulators that protect host tissues. Foreign surfaces that either lack membrane-bound regulators or cannot bind soluble regulators are attacked by complement.

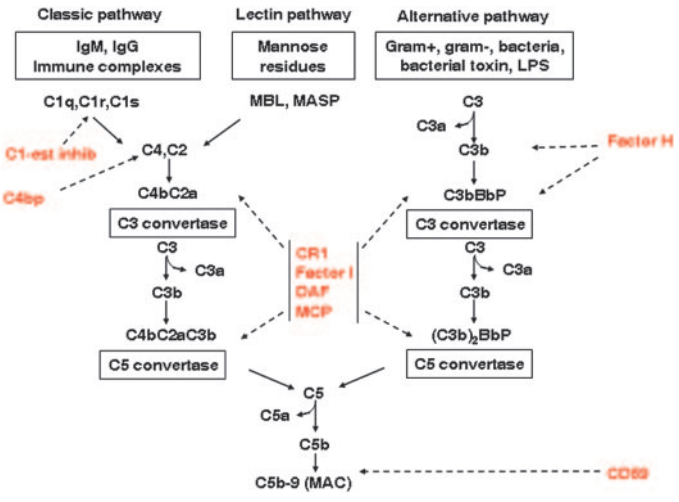

Figure 2. Activation pathways of the complement system and their regulators (in red).

In 1998, Warwicker *et al.* (110) studied three families with HUS and established linkage in the affected individuals to the regulator of complement activation gene cluster on human chromosome 1q32, which encodes for several complement regulatory proteins. The first examined candidate gene in this region was factor H (HF1), because an association between familial HUS and HF1 abnormalities had been reported previously (103,111,112). HF1 is a 150-kD multifunctional single-chain plasma glycoprotein that plays an important role in the regulation of the alternative pathway of complement (113). It serves as a co-factor for the C3b-cleaving enzyme factor I in the degradation of newly formed C3b molecules and controls decay, formation, and stability of the C3b convertase C3bBb. HF1 consists of 20 homologous units, named short consensus repeats (SCR). The complement regulatory domains that are needed to prevent fluid-phase alternative pathway amplification have been localized within the N-terminal SCR1–4 (114). The inactivation of surface-bound C3b is dependent on the binding of the C-terminal domain of HF1 to polyanionic molecules that increases HF1 affinity for C3b and exposes its complement regulatory N-terminal domain. The C-terminal domains contain two C3b binding sites, located in SCR12–14 and SCR19–20, and three polyanion-binding sites, located in SCR 7, SCR 13, and SCR19–20 (Figure 3) (115–117). However, the C3b and the polyanion-binding sites located in SCR19–20 are the only indispensable sites for HF1 to inactivate surface-bound C3b, because deletion of this portion of the molecule causes loss of HF1 capability to prevent complement activation on sheep erythrocytes (115,116). Human glomerular endothelial cells and kidney glomerular basement membrane are rich in polyanionic molecules, so HF1 deposited on their surface would provide an efficient shield against complement attack (Figure 4A) (117,118).

Since the first report by Warwicker, a number of studies have been performed by four independent groups, who altogether so far have identified up to 50 different HF1 mutations (Figure 3) in 80 patients who had familial (36 patients) and sporadic (44 patients) forms of non–Stx-HUS (99–102,114,119–121). In sporadic forms, the mutation was either inherited from a healthy parent or, more rarely—only four cases reported—ensued *de novo* in the proband (100,102). The mutation frequency is up to 40% in familial forms, whereas only 13 to 17% of sporadic forms had HF1 mutations (100,119). Alterations in other genes encoding for complement regulatory proteins could theoretically be involved in determining predisposition to sporadic non–Stx-HUS. Alternatively, these forms could be caused by an acquired autoimmune HF1 defect, similar to that observed in some patients with thrombotic thrombocytopenic purpura, in whom the acute episode is triggered by antibodies against the von

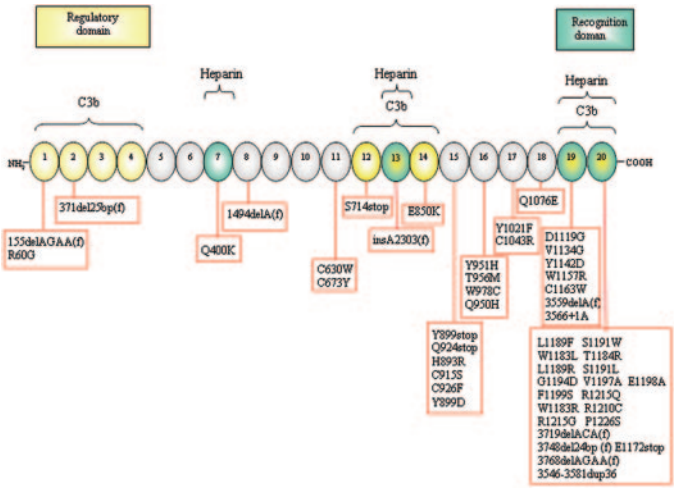

Figure 3. Factor H mutations associated with hemolytic uremic syndrome (HUS). The figure shows the structure of human factor H with the 20 short consensus repeats. The locations of the N-terminal regulatory domain responsible for co-factor activity and the binding sites for C3b and polyanions (heparin) are indicated. The majority of the mutations found in patients with HUS clusters in the C-terminus of factor H that is important for binding to polyanions and to surface-bound C3b and for the control of C3b deposition on cell membranes and extracellular matrix.

Willebrand factor cleaving metalloprotease ADAMTS-13 (122). This possibility is supported by a recently published paper (123) documenting the presence of anti-factor H antibodies in the plasma of three children with recurrent HUS.

The vast majority (48 of 50) of HF1 mutations in HUS patients are heterozygous and cause either single amino acid changes or premature translation interruption, mainly clustering in the C-terminus domains and are commonly associated with normal HF1 plasma levels. This is at variance with patients with type II membranoproliferative glomerulonephritis, who carry homozygous HF1 mutations that cause severely reduced HF1 levels (101). Expression and functional studies demonstrated that HF1 proteins that carry HUS-associated mutations have a severely reduced capability to interact with polyanions and with surface-bound C3b (117,118,124), which results in a lower density of mutant HF1 molecules bound to endothelial cells surface and a diminished complement regulatory activity on the cell membrane (117,118). In contrast, these mutants have a normal capacity to control activation of the complement in plasma, as indicated by data that they retain a normal co-factor activity in the proteolysis of fluid-phase C3b (124). The latter finding explains the case of patients who have HUS and HF1 mutations and normal serum complement levels (100,101). Sánchez-Corral *et al.* (125) proposed that HF1-related complement regulatory defects could be detected in patients' serum with an *ex vivo* hemolytic assay, in which serum from patients with HF1 mutations caused a more severe lysis of sheep erythrocytes than serum from patients without mutations. This, if confirmed, could represent a useful tool to select patients who have HUS and deserve studies of HF1 and other complement regulatory proteins.

Patients who carry HF1 mutations have a partial HF1 deficiency, as a result of one intact and one defective allele, which more likely predispose to rather than directly cause the disease. The observation that these patients occasionally have long remissions from HUS or do not present until late in life supports this hypothesis (125). In addition, conditions that trigger complement activation, either directly (bacterial and viral infections) or indirectly, by causing endothelial insult (drugs, systemic diseases, or pregnancy), precipitate the acute event in approximately 60% of patients with HF1 mutations (99,119). All of the above observations can be reconciled by reasoning that in these patients, the suboptimal HF1 activity is enough to protect the host from complement activation in physiologic conditions. However, upon exposure to an agent that activates complement, C3b is formed in higher-than-normal amounts, and its deposition on vascular endothelial cells cannot be fully prevented as a result of loss of polyanion binding capability of mutated HF1 (Figure 4B). This results in the formation of membrane attack complex and the recruitment of inflammatory cells, all events that cause damage and retraction of endothelial cells, adhesion and aggregation of platelets, increased local tissue factor with factor VII binding and activation, and the formation of thrombin and of fibrin polymers (Figure 4D). Such a scenario particularly applies to glomerular capillary bed, which is a fenestrated endothelium, and the exposed basement membrane supplies a surface that is rich in polyanions for HF1 binding, which could explain the renal localization of microvascular injury of HUS.

Two thirds of patients with non–Stx-HUS have no HF1 mutations, despite that up to 50% of them exhibit evidence of overactivity of the alternative pathway of complement (99). The possibility that uncommon polymorphic variants of HF1 gene may confer susceptibility to HUS in patients without HF1 mutations has been recently raised. Indeed, the T allele of the C-257T, the G allele of the A2089G, and the T allele of the G2881T polymorphisms were found to be more frequent in HUS patients without HF1 mutations than in healthy subjects (99), and analysis of the overall study population revealed that individuals who carry two or three of the above variants had a fourfold increased risk for developing HUS (99). The −257T, 2089G, and 2881T alleles might also have a role in determining the penetrance (which is approximately 50%) (99) of the disease in HF1 mutation carriers. In five of nine families, individuals who developed HUS had inherited an allele carrying the HF1 mutation from one parent together with an allele carrying at least one disease-associated HF1 polymorphism from the other parent. Instead, all of the healthy HF1 mutation carriers inherited only the mutation but no polymorphism (99).

Abnormalities in two additional genes encoding for complement modulatory proteins have also been involved recently in predisposition to non–Stx-HUS. Two reports from independent groups, published a few days apart, described mutations in MCP gene, encoding for membrane co-factor protein, a cell-bound complement regulator, in affected individuals of four families (127,128). MCP is a widely expressed transmembrane

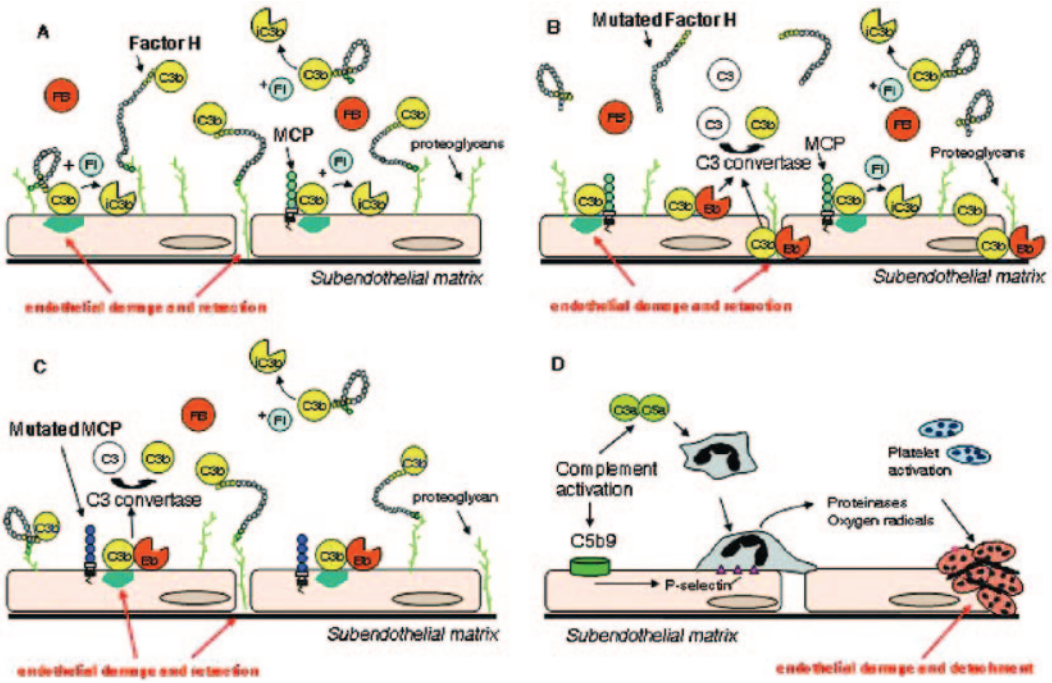

Figure 4. Proposed model for the pathologic consequences of factor H and monocyte chemoattractant protein (MCP) mutations. (A) After viral or bacterial infection or endothelial insult, complement is activated and C3b is formed. In the presence of normal factor H (HF1), C3b is rapidly inactivated to inactive C3b (iC3b). Factor H in the circulation binds fluid-phase C3b and favors its degradation by factor I (FI; co-factor activity, yellow domain). In addition, it binds (green domain) to polyanionic proteoglycans that are present on endothelial cell surface and in the subendothelial matrix, where, because of its high affinity for C3b, it entraps fluid-phase C3b, thus preventing its deposition on host surfaces and its binding with factor B (FB) to form the C3 convertase complex (C3bBb). The subendothelial matrix lacks endogenous complement regulators and is completely dependent on factor H to control complement activation. MCP also inactivates C3b deposited on endothelial cells by favoring its cleavage to iC3b by FI. (B) Proposed consequences of factor H mutations found in patients with HUS. Mutant factor H has a normal co-factor activity in fluid phase. However, the mutations affect the polyanion interaction site at the C-terminus of factor H so that it shows reduced bind to proteoglycans on endothelial cell surface and in subendothelial matrix. This results in more C3b reaching the endothelia cell surface so that MCP is not enough to control adequately complement activation on the cell membrane. In addition, C3b deposited on exposed extracellular matrix is not degraded and forms the C3 convertase of the alternative pathway of complement that further cleaves C3 to C3b. (C) MCP deficiency also predisposes to HUS. MCP mutations found in patients with HUS result in a reduced surface expression of the protein or in a reduced capability of MCP to bind C3b. In both cases, membrane-bound C3b is not efficiently inactivated, which leads to undesirable amplification of C3b formation and deposition on damaged endothelial cells through the formation of C3 convertase. (D) The sequence of events leading to microvascular thrombosis. The proteolysis of C3 and C5 by convertases causes the release of the chemotactic anaphylatoxins C3a and C5a that bind to receptors on inflammatory cells and attract them toward the endothelial layer. The deposition of C3b on endothelial cells is followed by the formation of the membrane attack complex (C5b9), which leads to cells' injury and detachment and to sublytic membrane perturbation, leading to endothelial activation and expression of adhesion molecules (*e.g.*, P-selectin). The latter favor leukocyte attachment and activation with the release of oxygen radicals and proteinases that further damage the endothelium. After endothelial damage, cell detachment ensues and exposes basement membranes. In these conditions, platelets from the microcirculation adhere and aggregate to the exposed matrix.

glycoprotein that serves as a co-factor for factor I to cleave C3b and C4b deposited on host cell surface (129–131). MCP has four extracellular complement control-protein modules (CCP) that are important for its inhibitory activity, followed by a serine-threonine-proline–rich domain, a transmembrane domain, and a cytoplasmic tail (132). Richards *et al.* (128) reported a heterozygous deletion of the D237/S238 amino acids in one family and a S206P substitution in two families. Evaluations of protein expression and function on PBMC showed that the mutants had a reduced C3b binding capability and a reduced ability to prevent complement activation. Another heterozygous mutation, causing two amino acid changes and a premature interruption of MCP protein in CCP4, was identified (127) in two siblings, which caused 50% reduction in MCP expression levels on PBMC of heterozygous individuals. Additional studies from our group on 112 patients with non–Stx-HUS have revealed five additional MCP mutations in familial (seven cases) and in sporadic (five cases) HUS with a mutation frequency of 11% (25% in familial and 6% in sporadic forms) (133).

MCP is highly expressed in the kidney and could be found on glomerular endothelial cells by immunohistochemical analysis (134–136). It likely exerts a main role in protecting glomerular endothelial cells against C3 activation as indicated by data that co-factor activity in the extracts of these cells was com-

pletely blocked by anti-MCP antibody (136). Factor H and MCP likely integrate each other in controlling complement activation on host cells. Polyanion-attached HF1 extends from the cell membrane by approximately 120 nm and could represent an outer barrier of cells against complement attack. However, because MCP is a small-sized membrane-integrated complement regulator that extends for approximately 20 nm, one can hypothesize that MCP protein is involved in the control of complement in the close vicinity of the cell membrane (117). As hypothesized for HF1, mutations in MCP likely predispose rather than directly cause HUS. Upon exposure to conditions that cause activation of the complement cascade, reduced levels or defective C3b binding capability and co-factor activity of mutated MCP on glomerular endothelial cells would result in an insufficient protection of these cells from complement activation (Figure 4C). That mutations either in factor H or in MCP result in complement activation and HUS indicates that these complement regulators do not have overlapping functions and that they both are necessary to control complement adequately.

Finally, three mutations in the gene encoding for factor I have been reported in three patients with sporadic non–Stx-HUS (137), which further support the concept that HUS is a disease of complement dysregulation. Other candidate genes are under investigation, including DAF, CR1, CD59, C3, and factor B.

Patients who present with non–Stx-HUS should be tested first for serum C3 concentrations; however, normal C3 levels do not necessarily exclude a complement dysfunction. More sensitive assays could be a higher-than-normal C3d/C3 ratio in plasma (unpublished data) or the presence of C3 deposits in renal biopsy (106,107). Measurement of HF1 in serum would be helpful to find out those few patients who carry HF1 mutations that cause reduced HF1 levels. Decreased CH50 values and factor B concentrations could be found in some but not all patients with HF1 or MCP mutations. A second step should be the search for mutations in candidate genes HF1 and MCP. Search for factor I mutations should be performed in patients with lower-than-normal factor I serum levels.

Which Treatment for Non–Stx-HUS?

Despite that non–Stx-HUS has a poor prognosis, after plasma manipulation was introduced, the mortality rate has dropped from 50 to 25% (138–140). However, debate still exists on whether plasma is or is not effective in the treatment of acute episodes (141–144). Published observations (139,145–147) and our own experience indicate that a consistent number of patients with non–Stx-HUS respond to plasma treatment. It has been proposed that plasma exchange might be relatively more effective than plasma infusion because it might remove potentially toxic substances from the patient's circulation. That this may not be the case is documented by data that in a patient with relapsing thrombotic microangiopathy (148), normalization of the platelet count was invariably obtained by plasma exchange or infusion, whereas plasma removal and substitution with albumin and saline never raised the platelet count. However, in situations such as renal insufficiency or heart failure, which limit the amount of plasma that can be provided with infusion alone, plasma exchange should be considered as first-choice therapy (1). Plasma treatment should be started within 24 h of presentation as delay in treatment initiation may increase treatment failure. Usually one plasma volume (40 ml/kg) is exchanged per session (1,149). Treatment can be intensified by increasing the volume of plasma replaced. The twice-daily exchanges of one plasma volume is probably the treatment of choice for refractory patients to minimize the recycling of infused plasma (1). As for plasma infusion, the recommended dose is 30 to 40 ml/kg on day 1, then 10 to 20 ml/kg per d. Daily plasma therapy should continue for a minimum of 2 d after complete remission is obtained (1,149).

Plasma infusion or exchange has been used in patients with HUS and HF1 mutations, with the rationale to provide the patients with normal HF1 to correct the genetic deficiency. Some patients did not respond at all and died or developed ESRD (107). Others remained chronically ill (121,150) or required infusion of plasma at weekly intervals to raise HF1 plasma levels enough to maintain remission (151). Stratton *et al.* (152) were able to induce sustained remission in a patient who had HF1 mutation and developed an acute episode of HUS and required hemodialysis. After 3 mo of weekly plasma exchange in conjunction with intravenous immunoglobulins, the patient regained renal function, dialysis was withdrawn, and plasma therapy was stopped. At 1 yr after stopping plasma therapy, the patient remained disease-free and dialysis independent. Plasma therapy is instead contraindicated in patients with HUS induced by *S. pneumoniae*, because adult plasma contains antibodies against the Thomsen-Friedenreich antigen, which may exacerbate the disease.

In those few patients with extensive microvascular thrombosis at renal biopsy, refractory hypertension, and signs of hypertensive encephalopathy, when conventional therapies including plasma manipulation are not enough to control the disease (*i.e.*, persistent severe thrombocytopenia and hemolytic anemia), bilateral nephrectomy has been performed with excellent follow-up in some patients (153). Other treatments, including antiplatelet agents, prostacyclin, heparin or fibrinolytic agents, steroids, and intravenous immunoglobulins, have been attempted, with no consistent benefit (1).

Patients who develop HUS upon challenge with cyclosporine or tacrolimus have to stop the medication. Sirolimus has been used as an alternative in occasional patients with encouraging results (154).

Of patients with non–Stx-HUS, 50% (in sporadic forms) to 60% (in familial forms) progress to ESRD (1,99). Renal transplantation is not necessarily an option for non–Stx-HUS, at variance with Stx-HUS. Actually, approximately 50% of the patients who had a renal transplant had a recurrence of the disease in the grafted organ (86,155). Recurrences occur at a median time of 30 d after transplant (range, 0 d to 16 yr). There is no effective treatment of recurrences. Graft failure occurs in >90% of patients who experience recurrence, despite plasma infusion or plasma exchange, high-dose prednisone, and withdrawal of cyclosporine (1,86). Patients who lost the first kidney graft for recurrence should not receive another transplant. Live-related renal transplant should also be avoided in that it carries the additional risk to precipitate the disease onset in the healthy

donor relative as recently reported in two families (156). New knowledge from genetic studies will predict more accurately the risk for recurrence. In patients with HF1 mutations, the recurrence rate ranges from 30 to 100%, according to different surveys (99–101), and is significantly higher than in patients without HF1 mutations (99). In view of the fact that HF1 is a plasma protein mainly of liver origin, a kidney transplant does not correct the HF1 genetic defect (110,119).

Simultaneous kidney and liver transplant was performed in two young children with non–Stx-HUS and HF1 mutations, with the objective of correcting the genetic defect and preventing disease recurrences (157,158). However, for reasons that are currently under evaluation and that possibly involve an increased liver susceptibility to immune or ischemic injury related to uncontrolled complement activation, both cases that were treated with this procedure were complicated by premature irreversible liver failure. In the first published case (157), a humoral rejection of the liver graft manifested by the 26th day after transplantation; the patient had actually a high titer of antibodies to donor class I HLA. In a few days, the child developed hepatic encephalopathy and coma that recovered with a second, uneventful liver transplant (157). The second case was complicated by a fatal, primary nonfunction of the liver graft. Graft hypoperfusion, as a result of a sudden drop of arterial BP occurring soon after reperfusion, triggered severe ischemia/reperfusion damage and complement deposition in the liver, conceivably as the result of defective HF1 complement regulatory potential. Multiorgan failure was the final event resulting in the patient's death (158). Thus, despite its capacity of correcting the genetic defect, combined kidney and liver transplant for non–Stx-HUS associated with HF1 mutations should not be performed unless a patient is at imminent risk for life-threatening complications.

Kidney graft outcome is favorable in patients with MCP mutations as found in four patients who received a successful transplant and experienced no disease recurrence (128; unpublished data). In view of the fact MCP is a membrane-bound protein that is highly expressed in the kidney, a kidney graft would reasonably correct local MCP dysfunction. The graft, bearing wild-type MCP expressed on renal endothelial cell surface, should conceivably be protected from disease recurrence.

The Future

Research efforts are aimed at identifying more specific approaches that may interfere with the primary cause of microangiopathy in the different forms of HUS. In Stx-HUS, new agents that are targeted at preventing organ exposure to Stx are currently under evaluation (159). In mice, molecular decoys such as orally administered harmless recombinant bacteria that display an Stx receptor on the surface that in turn binds the toxin in the gut (160–162) have been used successfully. Another approach is to use Stx inhibitors, among them is STARFISH, an oligobivalent, water-soluble carbohydrate ligand that can simultaneously engage all five B subunits of the toxin, which might help to prevent toxin that already has entered the circulation from destroying kidney microvessels (163). Others have ameliorated disease in pigs by injection of toxin-neutralizing antibodies (164). Some investigators have focused on downstream events in the pathogenetic cascade. Thrombin blockade with lepirudin in a model of Stx-HUS in greyhound dogs had some beneficial effect on mortality (165), indicating that thrombin may be a critical factor in the pathogenesis of Stx-HUS. At present, prevention remains the main approach to decreasing the morbidity and mortality associated with Stx-*E. coli* infection. A multifaceted approach that includes novel ways of decreasing Stx-*E. coli* carrier rate in livestock and implementing a zero-tolerance policy for contaminated foods and beverages is required.

The discovery of mutations in three different complement regulatory genes provides enough evidence of the involvement of complement activation in the pathogenesis of non–Stx-HUS and indicates that complement inhibition could represent a therapeutic target in these patients. There are currently a number of companies with complement inhibitors in clinical or preclinical development (166). Pexelizumab and eculizumab, two humanized monoclonal antibodies directed against C5 that inhibit the activation of terminal complement components, have been developed recently. Administration of eculizumab to patients with paroxysmal nocturnal hemoglobinuria, a disease characterized by a genetic deficiency of surface proteins that protect hematopoietic cells against the attack by the complement system, reduced intravascular hemolysis, hemoglobinuria, and the need for transfusions (167). In a phase II clinical trial, administration of pexelizumab as adjunctive therapy in patients who had myocardial infarction and underwent primary percutaneous coronary intervention inhibited complement activation and significantly reduced mortality as compared with the placebo group, although the infarct size was not modified by the drug (168). Another complement-blocking approach under investigation in clinical studies is based on the use of soluble forms of the C3/C5 convertase inhibitor complement receptor 1 (CR1). Phase I and phase II clinical trials have shown that the soluble CR1 TP10, administered intravenously both before and during surgery, decreased complement activation and protected vascular function in infants who underwent cardiopulmonary bypass (169). In a randomized, multicenter, prospective study in 564 high-risk patients who underwent cardiac surgery on cardiopulmonary bypass, a bolus of TP10 given immediately before cardiopulmonary bypass significantly inhibited complement activity within 10 min, and this inhibition persisted for 3 d postoperatively (170).

It is hoped that the above complement inhibitors, once available to the market, will be useful in patients with non–Stx-HUS, to block complement-mediated kidney damage during the acute episode or to prevent recurrence after kidney transplantation. Complement inhibitors also theoretically could be of benefit to prevent complications, such primary liver nonfunction, in combined kidney and liver transplantation in patients with HF1 genetic defects.

For HUS associated with HF1 mutations, specific replacement therapies with recombinant HF1 could become a viable alternative to plasma treatment. Efforts are also ongoing to isolate plasma fractions enriched in HF1, which could allow

providing the patient with enough active molecules while minimizing the risk for allergy and fluid overload. It is hoped that advances in vector safety and transfection efficiency will soon render gene therapy a realistic option for these patients. Undergoing studies on other complement regulatory genes would help to clarify fully the molecular determinants underlying the pathogenesis of non–Stx-HUS and hopefully translate into an improvement in the management and therapy.

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