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PERSPECTIVES IN BASIC SCIENCE

Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura

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Thrombotic microangiopathy, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. The term thrombotic microangiopathy (TMA) defines a lesion of vessel wall thickening (mainly arterioles or capillaries), intraluminal platelet thrombosis, and partial or complete obstruction of the vessel lumina. Depending on whether renal or brain lesions prevail, two pathologically indistinguishable but somehow clinically different entities have been described: the hemolytic uremic syndrome (HUS) and the thrombotic thrombocytopenic purpura (TTP). Injury to the endothelial cell is the central and likely inciting factor in the sequence of events leading to TMA. Loss of physiological thromboresistance, leukocyte adhesion to damaged endothelium, complement consumption, abnormal von Willebrand factor release and fragmentation, and increased vascular shear stress may then sustain and amplify the microangiopathic process. Intrinsic abnormalities of the complement system and of the von Willebrand factor pathway may account for a genetic predisposition to the disease that may play a paramount role in particular in familial and recurrent forms. Outcome is usually good in childhood, Shiga toxin-associated HUS, whereas renal and neurological sequelae are more frequently reported in adult, atypical, and familial forms of HUS and in TTP. Plasma infusion or exchange is the only treatment of proven efficacy. Bilateral nephrectomy and splenectomy may serve as rescue therapies in very selected cases of plasma resistant HUS or recurrent TTP, respectively.

The term thrombotic microangiopathy (TMA), first introduced by Symmers in 1952 [1], defines a lesion of vessel wall thickening (mainly arterioles or capillaries) with swelling or detachment of the endothelial cell from the basement membrane, accumulation of fluffy material in the subendothelial space, intraluminal platelet thrombosis, and partial or complete obstruction of the vessel lumina [2]. This lesion is common to various diseases [reviewed in 2]. Laboratory features of thrombocytopenia and hemolytic anemia are almost invariably present in patients with TMA lesions, and reflect consumption and disruption of platelets and erythrocytes in the microvasculature. Additional clinical signs depend on the diverse distribution of the microvascular lesions and the consequent organ dysfunction. Depending on whether renal or brain lesions prevail, two pathologically indistinguishable, but somehow clinically different entities have been described. They have been uniformly termed in the last few decades as hemolytic uremic syndrome (HUS), after the five children with hemolytic anemia, thrombocytopenia, and acute renal failure reported by Gasser et al in 1955 [3], or as thrombotic thrombocytopenic purpura (TTP), after the case of a 16-year-old female with acute febrile pleiochromic anemia, petechiae, paralysis, and coma reported by Moschowitz in 1923 [4]. The former syndrome usually affects young children; the latter occurs in adults. In the only comparative analysis of adult forms of HUS or TTP available so far, hematological and neurological abnormalities at disease onset were indistinguishable [5, 6]. Moreover, regardless of the severity of renal involvement, adults have similar outcomes that mostly depend on when treatment with plasma is effective in normalizing laboratory signs and inducing remission of clinical symptoms [5, 6]. To be practical and avoid confusion, here we first discuss children with HUS initiated by infections with Shiga toxin (Stx)-producing strains of *Shigella* or *Escherichia coli* infection (Stx-associated HUS). This form of HUS of children is separated from all the other forms of children HUS and from HUS and TTP of adults because the outcome and response to therapy is different in Stx-associated childhood HUS as compared with all the other forms of HUS and TTP [7]. As well, simply for practical reasons, for all forms of non-Stx–associated HUS or TTP, the broader term HUS/TTP [8] will be used.

ROLE OF ENDOTHELIAL INJURY: AN OLD IDEA STILL IN PLACE

Most authorities consider endothelial injury as the central and likely inciting factor that sustains the microangiopathic process (Fig. 1). This is not a recent idea, however. As early as in 1942, Mark Altschule of Harvard Medical School suggested that microvascular endothelial activation was the primary event causing platelet deposition

Key words: Shigatoxin, endothelial activation, von Willebrand factor.

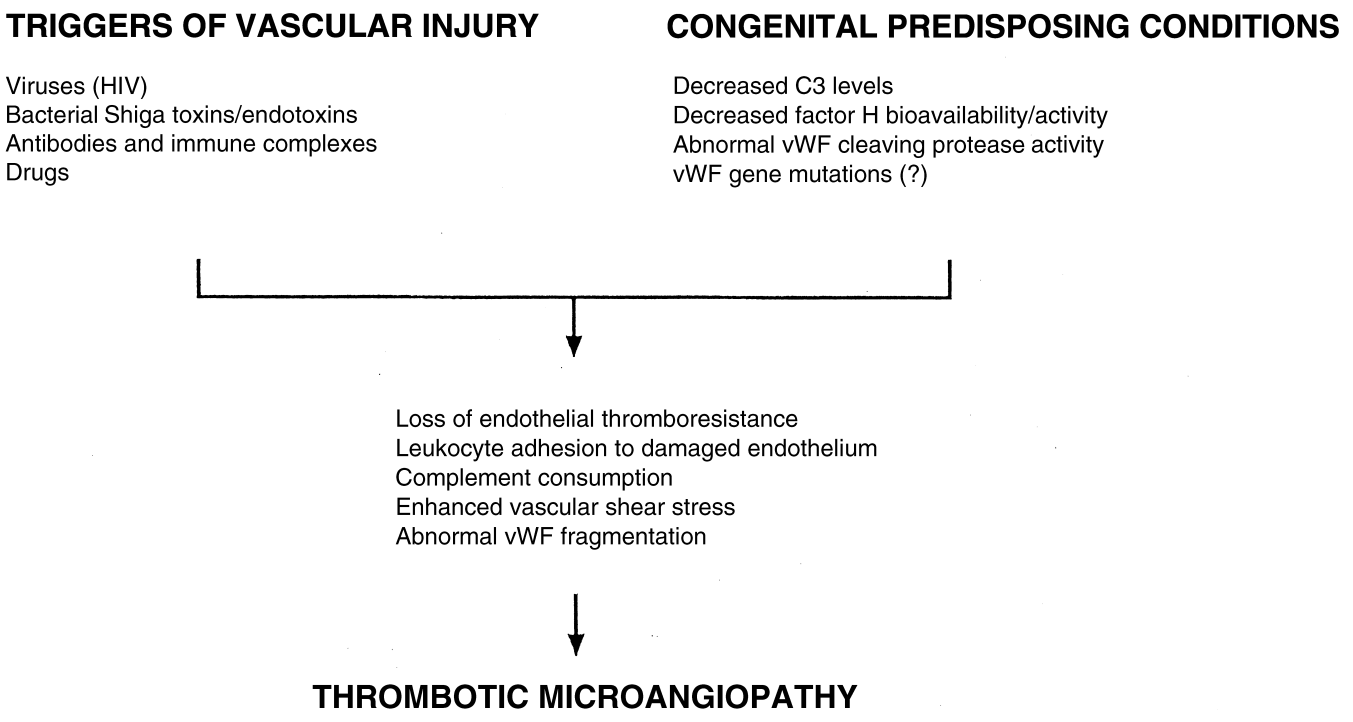

Fig. 1. A suggested sequence of events leading to thrombotic microangiopathy in predisposed individuals exposed to triggers of vascular injury. Genetic predisposition may have a predominant role in familial and recurrent forms, exposition to triggers of vascular injury in Shiga toxin *E. coli*-associated HUS.

in arterioles and capillaries with secondary "clearance of enormous numbers of platelets from the circulation" [9]. Actually, of the agents that have been associated with the disease in subsequent years, all are toxic to microvascular endothelium [reviewed in 2]. Moreover, plasma from patients with acute HUS/TTP induces apoptosis of human endothelial cells from the renal and cerebral microvasculature, but not from large vessels [10].

AGENTS IMPLICATED IN ENDOTHELIAL ACTIVATION AND DAMAGE

Shiga toxins

Despite that, the term HUS was introduced in 1954 [3]. A careful look at the older literature reveals some relevant observations. In 1927, Adam first reported that an infant epidemiology of gastroenteritis was associated with a special type of bacterium coli, biochemically unique for its fermentation properties [11]. Subsequent observations described a similar disease with diarrhea, occasionally complicated by purpura, anuria, and neurological signs, that was characterized at autopsy by capillary and arteriolar thrombosis and glomerular tuft occlusion by fibrin thrombi. *E. coli* serotype O111:B4 was considered the causative agent in more than 90% of these cases [12]. Further studies found a filterable agent isolated from the stools of these children that caused diarrhea in calves and was lethal to mice [12]. These findings were taken to indicate that a toxin possibly released by the microorganism could induce hemorrhagic necrosis of the gastrointestinal mucosa and—once absorbed into the blood stream—of other organs. Several years later, Konowalciuck, Speirs and Stavric noted that *E. coli* isolated from human cases with diarrhea, produced a toxin similar to the one of *Shigella dysenteriae* type 1 (Shiga toxin or Stx) found cytopathic to Vero cells (African green monkey kidney cells) [13]. This toxin was subsequently given different names such as Shiga-like toxin (SLT), or verotoxin. In 1978, Koster et al suggested that a circulating toxin was the cause of colitis, hemolysis, and renal failure in children HUS with evidence of *S. dysenteriae* type 1 infection [14]. A few years later, a case-control study by Riley et al found a strong association between hemorrhagic colitis and ingestion of *E. coli* O157:H7-infected beef patties at outlets of a well-known fast-food restaurant chain [15]. Recovery of the same strain of *E. coli* (which could be readily distinguished from other fecal *E. coli* because of its unique inability to ferment sorbitol) from the stools of about half the 47 children with diarrhea, but from none of the healthy controls, suggested a cause-and-effect relationship between food-borne *E. coli* infection and hemorrhagic colitis [15]. During the same period, Karmali et al demonstrated an increased Stx activity in fecal filtrates and increased Stx-neutralizing antibody titer in sera from children with

E. coli O157:H7 infection and sporadic, diarrhea-associated HUS [16]. Thus, almost 60 years after the seminal observations by Adams et al, these findings provided definitive demonstration of a direct role of a bacterial toxin (Stx) in the pathogenesis of HUS.

Thus far, more than 100 *E. coli* serotypes have been found to produce Stx, but only few of them have been involved in human disease. The serotype O157:H7 is by far the most common pathogen in the United States and Europe, but other strains, particularly the O111:H-serotype, are frequently reported in other areas of the world [17]. Of interest, *E. coli* O157:H7 strains that are isolated from patients with HUS usually produce both Stx-1 and Stx-2 or only Stx-2. Of note, in a recent epidemy of Stx-1 *E. coli* hemorrhagic colitis, no patient developed HUS, suggesting that Stx-2 *E. coli* is the involved pathogen at least in most of the cases (abstract; Hashimoto H, et al, *Pediatrics* 103:141, 1999) [18].

Escherichia coli O157:H7 colonizes healthy cattle intestine, but also has been isolated from deer, sheep, goats, horses, dogs, birds, and flies [19]. It is found in manure, water troughs, and other places in farms, which may explain the increased risk of infection observed in people living in rural areas. The microorganism is transmitted to humans by food and water, directly from person to person [20, 21] and occasionally through occupational exposure. Most food-borne outbreaks have been attributed to cattle-derived foods, in particular beef and raw milk, although contaminated foods from other sources, such as lamb and jerky, have been involved in some cases. Meat probably becomes contaminated at the time of slaughter, and internalization of the microorganism during grinding may render it more likely to survive cooking. Fruits and vegetables may also be contaminated, and radish sprouts, lettuce, apple cider, unpasteurized apple juice, and alfalfa sprouts have been implicated in several outbreaks. Water-borne outbreaks have occurred as a result of drinking and swimming in unchlorinated water. Person-to-person transmission has been reported in day-care and chronic-care facilities [reviewed in 17].

After oral ingestion of contaminated food or water, *E. coli* O157:H7 reaches the gut and closely adheres to the epithelial cells of the gastrointestinal mucosa. Adhesion by a 97 kD outer membrane protein, intimin, produces characteristic "attaching and effacing" lesions aimed at preventing the expulsion of the microorganism [22]. The consequent disruption of the brush border per se is sufficient to produce nonbloody diarrhea. In addition, however, *E. coli* O157:H7 (as well as other Stx-producing serotypes such as *E. coli* O111, nonmotile O26:H11, or O103:H2) may produce large amounts of Stx, which traverse polarized gastrointestinal epithelial cells, probably via transcellular pathways [23], and gains the systemic circulation. How the toxin is transferred from the intestine to target organs is still not known. Free toxin has never been detected in the circulation of patients with HUS. Of note, a very recent study found that, in vitro, Stx rapidly binds to polymorphonuclear leukocytes (PMNs), but not to erythrocytes, monocytes, platelets, or lipoproteins [24]. However, the Stx receptor on PMNs has a 100-fold lower affinity than the high-affinity receptor (globotriaosylceramide or Gb3) expressed on glomerular endothelial cells. As a consequence, at least in the in vitro cocultures, PMNs loaded with Stx transfer the ligand to glomerular endothelial cells, which promote cell death [24]. Should this occur in vivo, it would explain the rapid clearance of Stx from the circulation and its release to target organs where the toxin exerts its cytopathic effect.

Shiga toxins produced by *S. dysenteriae* type 1 and *E. coli* comprise a family of multisubunit toxins that are structurally and genetically related. Shiga-like toxins (SLTs) are classified according to the extent of antigenic similarity to Shiga toxin. SLTs whose cytotoxic activity is neutralized by antisera to Shiga toxin are referred to as type 1 (Stx-1), whereas SLTs that are not cross-neutralized by anti–Shiga-toxin antibodies are classified as type 2 (Stx-2). Subtypes of Shiga-toxin 2 (Stx-2c and Stx-2e) share cross-hybridization of their genes under conditions of high stringency, but show significant differences in biologic activity, serological reactivity, or receptor binding [25]. All members of the toxin family consist of pentamers of 7 to 8 kD binding subunits (B subunits) in noncovalent association with single enzymatic subunits (A subunits) of approximately 35 kD. They bind to target cells via the neutral glycolipid Gb_3 and globotetraosylceramide (Gb_4) and mediate protein synthesis inhibition in target cells by the specific N-glycosidase activity of A subunits that act at a site adjacent to adenine-4324 in the 28 S rRNA component of eukaryotic ribosomes. Human glomerular endothelial cells are sensitive to the cytotoxic effects of Stx, particularly when exposure to tumor necrosis factor-α (TNF-α) increases Gb3 expression on cell surface and Gb3-mediated toxin binding and internalization by endocytosis [26]. Gb3 fatty acid composition and phospholipid chain length within the phospholipid bilayer also may play an important role in the intracellular distribution and biological effects of the toxin. For instance, Gb3 receptors containing shorter chain fatty acid species direct the toxin to the endoplasmic reticulum/rather than to the Golgi apparatus, and increase Stx cytotoxicity [27]. Genetic differences in Gb3 fatty acid composition might therefore account for different individual susceptibility to the effects of Stx [27]. Inhibition of protein synthesis and cell death are the usual effects of Stx. Sublethal Stx doses also may alter the production of endothelial-derived vasoactive mediators such as endothelin and nitric oxide, which, in turn, might contribute to the microvascular changes characteristic of hemorrhagic colitis and HUS. Moreover, sublethally injured endothelial cells have an increased susceptibility to injury mediated by activated PMNs [28]. Studies have shown that

up-regulation of cytokine production, PMN activation, and enhanced oxidant injury potentiate the direct cytopathic effects of Stx; this amplifies vascular damage and, conceivably, alters the normal thromboresistant phenotype of the endothelial cell [reviewed in 29].

However, endothelial cells are not the only targets for Stx in the kidney, and several renal cell types (such as proximal and distal tubular cells or mesangial and epithelial glomerular cells) have receptors for Stx and are damaged by the toxin in vitro [30]. These findings suggest that renal injury in Stx-HUS, unlike other forms of HUS/TTP, is not simply a consequence of vascular involvement in the microangiopathic process, but may reflect, at least in part, a primary damage to parenchymal cells [30].

Experimental evidence indicates that sites of Gb3 expression coincide with sites of Stx-induced tissue damage [2]. Indeed, after a challenge with Stx-1 purified from *E. coli* O157:H7, rabbits presented neurologic and enteric features of endothelial swelling and luminal occlusion of small arteries and arterioles, but no signs of renal involvement [31]. Binding studies identified Stx receptors in the central nervous system and in the gastrointestinal tract, but not in the kidney [31]. In humans, the Gb3 receptor is expressed not only in the kidney, but also on the membrane of epithelial and endothelial cells of gastrointestinal mucosa and submucosa, respectively. Of interest, a recent study in baboons, which share with humans a similar distribution of gastrointestinal and renal Gb3 receptors, demonstrated that Stx injection induces direct injury to the endothelial and epithelial cells of the gastrointestinal and renal microcirculation [32]. In this animal model, structural changes reminiscent of the autoptic changes observed in children with HUS and neurological involvement also were detected in the central nervous system, despite a relatively low expression of the Gb3 receptor in this organ [32]. Increased local release of inflammatory mediators was advocated to contribute to renal tissue injury. This possibility is supported by the observation that neurological changes can be associated with increased interleukin (IL)-6 concentration in liquor. Thus, the finding that, despite negligible Gb3 receptor expression, adult humans may develop central nervous system lesions secondary to Stx-producing *E. coli* infection [20, 33] could be due to a facilitating effect of enhanced local release of inflammatory mediators [31, 32].

Endotoxins

Upon *E. coli* infection, bacterial-derived endotoxins (lipopolysaccharides or LPS) and Stx act synergetically in initiating damage to target organs, including the kidney. LPS prime the endothelial cells to undergo apoptosis when exposed to picomolar amounts of Stx. Moreover, LPS derived from Stx producing *E. coli* activate PMNs to release TNF-α, IL-1, elastase, and free radicals that are highly toxic to microvascular endothelium [34].

Bacterial neuraminidases

Following a few reports in the European literature, Klein et al found Thomsen-Friedenreich antigen on erythrocytes and in the glomeruli of two one-year-old children who died from HUS secondary to pneumococcus pneumonia and sepsis [35]. Binding studies also found weak IgM deposits in the glomeruli indicative of a specific IgM binding to the Thomsen-Friedenreich antigen. Subsequent studies demonstrated neuraminidase activity in organism-free filtrates of sera from three infants with *Streptococcus pneumoniae*-associated HUS, but not in filtrates of sera from a large series of samples from children with *Streptococcus pneumoniae* infection and no evidence of HUS. Since the Thomsen-Friedenreich antigen is normally covered by *N*-acetyl neuraminic acid (sialic acid) moieties, it has been speculated that *Streptococcus pneumoniae*-derived neuraminidase, by removing sialic acid from the cell membranes, exposes this cryptic antigen to preformed circulating IgM antibodies [35, 36]. Then, IgM binding to Thomsen-Friedenreich antigen exposed on platelet and endothelial cell surface would cause platelet aggregation and endothelial damage [35, 36]. Binding of IgM antibodies to the antigen expressed on circulating erythrocytes may explain why Coombs-positive hemolytic anemia is so frequently reported in patients with neuraminidase-induced HUS.

Immune complexes

Complement-dependent antibodies, which are cytotoxic endothelial cells, have been found in the serum and plasma of patients with HUS/TTP [37]. Complement and IgG and IgM antibodies can be found in kidney biopsies [2]. Together, these findings suggest a pathogenetic role for immune complexes. This possibility is consistent with findings that endothelial cells exposed in vitro to sera of patients with HUS/TTP in the presence of complement show ultrastructural changes of cytoplasmic inclusions and cytoplasmic and nuclear degeneration suggestive of endothelial cell apoptosis [37].

Hemolytic uremic syndrome/TTP complicates immune diseases, in particular systemic lupus erythematosus, and increasingly is reported in association with the antiphospholipid syndrome [reviewed in 38].

Drugs

Several chemotherapeutic agents, including mitomycin C (MMC), cisplatin, daunorubimicin, cytosine arabinoside, chlorozotocin, neocarcinostatin, and deoxycoformycin, have been implicated in a syndrome of thrombotic microangiopathy designated as chemotherapy-associated HUS (C-HUS) [reviewed in 39]. MMC causes TMA after direct infusion into rat kidneys. Endothelial cells metabolize MMC to a reactive intermediate that cross-links cellular DNA and proteins. Endothelial cells, ex-

posed to MMC in vitro at concentrations equivalent to therapeutic drug levels in vivo, survive but subsequently show both an impaired ability to divide and a reduced thrombin-stimulated production of prostacyclin.

Cyclosporine. Cyclosporine A (CsA) has been associated with de novo thrombotic microangiopathy in renal and nonrenal transplantation and in patients with Behçets disease [40]. Thrombotic microangiopathy in renal allograft recipients is most common in the first weeks, when CsA drug levels are highest, and should be differentiated from both humorally and/or cell-mediated rejection. In renal allograft recipients, CsA enhances platelet aggregation and thromboxane A_2 production, and high CsA levels correlate with increased thrombomodulin and von Willebrand factor serum levels. Since the first report by Schmidt et al in 1991, more than 20 cases have been described providing evidence that tacrolimus may cause HUS/TTP [41, 42]. The disease is reported in 1 to 4.7% of tacrolimus treated patients. It is more frequently diagnosed in the first year after transplantation, although it may occur at any time.

Ticlopidine and clopidogrel. Ticlopidine has been associated with the development of HUS/TTP, but this event is rare (1 in 1600 patients treated with ticlopidine after cardiac stenting) [43]. Clopidogrel, a new antiplatelet agent that has achieved widespread clinical acceptance because of a more favorable safety profile than ticlopidine, also has been associated with the disease, and 11 cases have been reported recently [44]. Ten of these patients developed TTP within two weeks from beginning the drug treatment. Ticlopidine and clopidogrel are structurally related derivatives of thienopyridine and act by blocking an adenosine diphosphate-binding site on platelets, which inhibits the expression of the glycoprotein IIb/IIIa receptor in the high-affinity configuration that binds fibrinogen and large von Willebrand factor (vWF) multimers. Why these two drugs cause TTP is not fully clear, but of great interest, as patients with ticlopidine- or clopidogrel-associated TTP have a deficiency of vWF-cleaving protease activity in plasma that appears quite comparable to the deficiency observed in idiopathic TTP [44].

Quinine. Quinine is widely used for the prophylaxis of nocturnal cramps, but is also present in tonic water and bitter lemon drinks. Occasionally, it may cause hemolytic anemia, thrombocytopenia, disseminated intravascular coagulation, and HUS [45]. HUS typically occurs in patients presensitized by prior exposure to quinine and rapidly follows re-ingestion of the compound. Quinine-dependent antibodies to red blood cells, granulocytes, and the glycoprotein Ib/IX and IIb/IIIa on platelet surface have been involved in the pathogenesis of the disease. Renal failure is usually severe, but the outcome is good, provided that patients are treated early enough with plasma exchange [46].

CONSEQUENCES OF VASCULAR INJURY

Virtually all properties of the normal microvascular endothelium are altered in HUS/TTP. Endothelial cells synthesize many substances involved in coagulation and fibrinolysis, including prostacyclin, nitric oxide (NO), vWF, thrombomodulin, tissue-type plasminogen activator inhibitor and protein S. Loss of prostacyclin [47] and increase in vWF [48] have been claimed to account for the loss of physiologic thromboresistance and for the consequent widespread platelet aggregation in vascular beds throughout the body, creating a cycle of vasoconstriction with platelet and fibrin deposition and further thrombus formation.

Leukocyte activation and complement consumption

The interaction between leukocytes and damaged endothelial cells is instrumental to the development of microvascular injury in Stx-associated HUS, as documented by experimental and clinical evidence [34]. Neutrophils from children in the acute phase of HUS adhere to endothelial cells in vitro more tightly than normal neutrophils and induce endothelial injury by degrading endothelial cell fibronectin, possibly by the release of neutrophil-specific proteases [34]. Stx in vitro promotes massive leukocyte adhesion and transmigration to endothelium under flow conditions by up-regulating endothelial expression of adhesive proteins and chemokines (abstract; Zoja et al, *J Am Soc Nephrol* 10:595A, 1999). Platelet and leukocyte activation concur to promote the formation of C3bBb convertase, which in turn leads to cleavage of the third fraction of complement (C3) to the active form C3b, ready to react with any cell surface or membrane nucleophile. Low C3 levels were first described in diarrhea-associated HUS in the early 1970s [49]. Persistently low complement levels were found in different forms of HUS [49]. Granular C3 deposits in glomeruli and arterioles of patients with either diarrhea-associated or adult forms of HUS/TTP and evidence of C3 breakdown products in patients' sera were taken to reflect complement consumption in the microvasculature [50].

Abnormal von Willebrand factor release and processing

In normal individuals, vWF is formed as large multimers [ultralarge (UL) multimers] due to the polymerization in endothelial cells and megakaryocytes of a native subunit with an apparent molecular mass of 225 kD, and is stored as such in Weibel-Palade bodies and platelet granules. UL multimers do not normally circulate, since they are rapidly reduced into smaller multimers soon after their secretion by cleavage at position 842 Tyr-843 Met of the mature subunit. A major contribution to the understanding of vWF processing was provided by Furlan et al [51] and Tsai and Lia [52], who partially purified

and characterized a plasma metalloprotease that physiologically cleaves ultralarge vWF multimers. The protease, of approximately 300 kD, needs bivalent cations for its activation, is inhibited by calcium-chelating agents, and is activated only in conditions of low ionic strength or high shear stress. In vivo evidence that proteolytic cleavage is involved in the modification of plasma multimers after secretion has been provided by studies showing that circulating vWF multimers are heterogeneous oligomers of a native 225 kD subunit and of proteolytic fragments with apparent molecular masses of 189, 176, and 140 kD [53]. In patients with HUS and TTP, in contrast to healthy subjects, UL multimers similar to the ones stored in endothelial cells and platelets were occasionally detected in plasma [54]. The presence in patients with HUS/TTP of circulating UL multimers that in vitro are capable of supporting platelet aggregation more efficiently than normal multimers was evidence for their pathogenic role in microvascular thrombi [55]. However, direct proof that this was indeed the case was never provided. Interestingly, the opposite appears true in vivo, since the available evidence is against the possibility that UL vWF multimers when present in the circulation can even cause intravascular thrombosis. UL multimers have been found in fetuses and newborns, but disappear within the first months of life, indicating that physiologic cleavage of vWF is less efficient at birth and fully develops later in life [56]. Moreover, in patients with a rare variant of von Willebrand disease, named "Vicenza," UL vWF multimers were constantly found in the circulation; nonetheless, these patients never experienced thrombotic episodes; rather, they suffered with bleeding tendency [57].

In patients with HUS/TTP who recovered after a single episode, UL multimers were found almost exclusively in the acute phase but not in remission, suggesting a massive release from storage sites of acutely injured endothelial cells that possibly transiently overwhelmed the plasma proteolytic capacity [58]. In contrast, those cases that had a tendency to recur had circulating UL multimers either in the acute phase or consistently in the remission phase of the disease, which was initially taken as evidence of a state of persistent endothelial perturbation [54]. The hypothesis that circulating UL vWF multimers may reflect a condition of endothelial perturbation has been recently challenged by findings that circulating UL multimers in chronic relapsing TTP were associated with a reduced or totally absent activity of the above vWF-cleaving protease activity that normally cleaves vWF multimers to smaller molecular forms. In two large studies, vWF-cleaving protease deficiency was described in patients with different forms of TTP [51, 52]. In familial forms of TTP, the deficiency is probably inherited as an autosomal recessive trait. Consistent with this possibility, complete deficiency of vWF-cleaving protease activity was found in two brothers with chronic relapsing TTP, whereas their parents had approximately half-normal protease activity [59]. Of interest, in both patients with constitutional protease deficiency, disease remission achieved by plasma therapy was concurrent with an almost full recovery of the vWF-cleaving protease activity. Both patients achieved a long-lasting remission, although protease activity decreased to less than 20% over 20 days after plasma therapy withdrawal.

In nonfamilial cases, the protease deficiency appears to be a consequence of a specific autoantibody that develops transiently and tends to disappear during remission [51]. In a patient with recurrent episodes of TTP shown to have an acquired inhibitor of the vWF-cleaving protease [60], splenectomy, performed one year after the first TTP event, resulted in the disappearance of the autoantibody and normalization of the protease activity, platelet count, and hemoglobin levels [60].

At variance with TTP, in the previously mentioned studies, no deficiency was found in patients with familial or sporadic forms of HUS [51], suggesting that the presence or absence of this activity were enough to classify patients as having HUS or TTP, respectively. More recent data, however, indicate that low or zero protease activity is not confined to TTP and can even be found in a number of diseases associated with an increased tendency to thrombosis, such as inflammatory states, liver cirrhosis, major surgery (Mannucci et al, personal communication, Workshop on vWF and TTP, Bethesda, July 31, 2000), and disseminated malignancies [7]. Another child who was diagnosed as HUS/TTP, although he had renal failure and no neurological symptoms, showed with zero vWF protease activity [61]. Our group has obtained similar results of complete lack of protease in two cases of recurrent HUS (unpublished results). These data indicate that the issue is controversial and the distinction between HUS and TTP is much more complicated than presented in the *New England Journal of Medicine* articles [51, 52] and accompanying editorial [55].

A constant finding in the acute phase of different forms of HUS/TTP is an increase of low molecular weight multimers and a decrease of high molecular weight multimers, which reflects an enhanced proteolytic fragmentation of the molecule [62, 63]. That vWF undergoes excessive fragmentation in the acute phase of these diseases is remarkably consistent with previous findings of a relative decrease in the native 225 kD vWF subunit, which only occurs in the acute phase, accompanied by a relative increase of fragments that can only derive from the cleavage of the native subunit [64].

Enhanced vascular shear stress as a determinant of abnormal von Willebrand factor fragmentation

von Willebrand factor's susceptibility to fragmentation increases in response to rising levels of shear stress [65], which induces protein unfolding and makes vWF proteolytic cleavage sites more accessible to specific plasma

protease(s). It is speculated that enhanced shear stress in the severely narrowed damaged microvessels accounts for the abnormal vWF fragmentation observed during the acute phase of HUS/TTP. Evidence of increased capacity of fragmented vWF to bind receptors on activated platelets suggests that shear stress-induced vWF fragmentation may contribute to maintain and further spread microvascular thrombosis. In severe forms of HUS that are resistant to plasma therapy, removal of the kidneys—a major site of vascular bed occlusion and augmented shear stress—was followed by hematologic and clinical remission associated with restoring the vWF fragmentation pathway to normal [62]. Consistent with this possibility in patients with recurrent and sporadic HUS/TTP, increased vWF fragmentation normalized after resolution of the microangiophatic process.

GENETIC PREDISPOSITION

Congenital complement abnormalities

Over the last 20 years, approximately 140 cases of familial HUS and TTP have been described in 70 families, with the predominant features of HUS in two thirds of the patients. Both autosomal-recessive and autosomal-dominant modes of inheritance have been recognized [66]. Precipitating events such as pregnancy, virus-like disease, or sepsis have been reported only in a minority of cases. Evidence that some of these cases responded, at least transiently, to plasma infusion or exchange, suggests that the genetic defect(s) associated with familial HUS/TTP were the cause of one or more abnormalities in plasma component(s) essential to the integrity of microvascular circulation and/or to the defense mechanism of the host endothelium against injurious agents. Thus, reduced serum levels of the third component (C3) of the complement system have been reported since 1974 [49] in both the sporadic and familial forms of HUS [67]. That the disease may be related to an inherited congenital abnormality is consistent with the finding that more than 50% of patients with familial HUS/TTP—as compared with only 10 to 20% in nonfamilial forms—had repeated recurrences. This genetical abnormality is likely to involve the complement system, as suggested by levels of circulating C3, which were extremely low in a large series of familial cases as compared with controls [67]. Reduced C3 levels in cases and case relatives, but not in controls and the controls' relatives, further indicate that the defect clusters in families. Evidence that a low C3 concentration was strongly associated with the disease even more convincingly suggests the possibility of a tight (possibly causal) relationship between decreased C3 and disease manifestation [67]. On the other hand, in the previously mentioned series, low C3 levels could not depend on consumption in a still-ongoing microangiopathic process, since no patient at the time of the study had any sign of acute disease, and only two had moderately increased LDH levels [67].

Actually, low C3 levels are well known to accompany the acute phases either of diarrhea-associated or idiopathic HUS [49] and likely reflect C3 consumption in the microvasculature. Granular C3 deposits in glomeruli and arterioles of HUS patients [50] and evidence of C3 breakdown products in HUS sera [49] further document the activation of the complement system in the acute phase of the disease. Enhanced release of complement cleavage products (including C3a and C5a) consequent to uncontrolled complement activation may contribute to the microangiopathic process by stimulating neutrophil activation phagocytic adhesion to vascular endothelium, or platelet aggregation, and by directly injuring the endothelium through enhanced production of the membrane attack complex, the final multimolecular unit of the complement C5b-9.

Two complement pathways can generate C3-activating enzymes: the classic convertase generated by the sequential reaction of C1, C4, and C2, and the alternative pathway convertase. Activation of classic and alternative complement pathway—possibly triggered by circulating immune complexes and damaged erythrocytes, respectively—is well documented in acute HUS [68], but consistently subsides with remission of the disease. In contrast, in cases of familial HUS/TTP, serum C3 levels were consistently and remarkably depressed in cases as compared with controls, even during remission of the disease [67]. Even more interestingly, low C3 levels also were found in the patients' relatives, who had never suffered with HUS or TTP in the past and had no sign of the disease at the time of the study [67]. Furthermore, either in cases or in case-relatives depressed C3 values did not parallel similar changes in C4 levels. These data definitely ruled out the possibility that classic pathway activation accounted for hypocomplementemia in this series [67]. An inherited defect in C3 synthesis has been suggested to account for decreased C3 serum concentration [67], but much more convincing data are now available that low C3 in HUS may derive from either a lack or altered function of factor H, a regulatory protein that inhibits the complement activation through the alternative pathway [69].

INHERITED FACTOR H DEFICIENCY

In one patient with HUS and in his healthy brother, a persistent reduction in C3 levels was found with very low levels of factor H [70]. Finding that the parents, who were first cousins, had half-normal levels of factor H, convincingly indicated that the defect was inherited. A similar finding was later reported in another family. In a recent report, the association between inherited factor H deficiency and low C3 levels was investigated in a large series of families with a history of HUS/TTP [67].

By radial immunodiffusion and Western blot analysis, two affected subjects of one family were identified who had very low circulating factor H levels, and moderately low levels were found in two healthy relatives. In these patients, the cofactor activity of factor H, measured as the capacity to degrade C3b, was also reduced. In the other families, serum factor H concentration was normal [67]; however, normal serum levels do not necessarily exclude an underlying biochemical abnormality in circulating factor H. In this regard, in another family the two affected members, and the healthy father, who had normal serum concentrations of factor H by radial immunodiffusion, Western blots showed additional bands of higher molecular weights that were not found in any controls [67]. The bands might represent dimeric forms of factor H. At variance, no differences were found in serum levels and patterns of FHL-1 and FHR proteins [69]. Similar results were obtained in another series of patients with HUS [71].

Factor H has several biological activities. It prevents the formation of C3bBb complex and accelerates the dissociation of Bb from the C3 convertase. It acts as a cofactor for factor I that degrades C3b [69], and it also distinguishes between activator and nonactivator surfaces [69]. Other less well-defined functions of factor H have been suggested by the presence of at least two heparin binding sites in factor H protein that could facilitate interaction with extracellular matrix [69]. An intercurrent exposure to agents potentially toxic to the vascular endothelium (such as certain virus, bacteria, toxins, immunocomplexes, and cytotoxic drugs [2]) may initiate a local intravascular thrombosis, which promotes C3bBb convertase formation and complement deposition within capillary vessels. In normal conditions, however, by modulating C3bBb activity factor H may effectively limit complement deposition and further extension of the process [69]. In contrast, when the bioavailability and/or activity of factor H is congenitally defective, C3bBb convertase formation and complement deposition may become uncontrolled, with further extension of the microangiopathic process up to full manifestation of the disease.

GENE MUTATIONS ACCOUNTING FOR FACTOR H DEFICIENCY OR ABNORMALITIES

The consistent association found in families between factor H abnormalities and low C3 levels supports the hypothesis that low C3 in the setting of familial HUS/TTP may depend on a genetic deficiency of factor H.

A recent report on three large families with HUS documented that an area on chromosome 1q, where factor H is mapped, segregates with HUS [72]. All subjects in the three families had normal serum factor H levels. However, affected members and obligate carriers within one family were found, by mutation analysis, to have a heterozygous point mutation in factor H, consisting of a C to G transversion causing an arginine to glycine change in the short consensus repeat 20 (SCR20) [72]. This mutation is likely to alter structure and hence function of factor H protein without modifying its circulating levels. The same authors also described a nonsense mutation, located in SCR1 of factor H gene, in a single case with late onset [72]. In a large series of patients, four new heterozygous mutations in factor H gene were found [73]. Three of the mutations were observed in families with dominant transmission, the fourth was found in a single case who experienced disease recurrence on the renal allograft. Loirat et al have recently reported five cases of atypical HUS with neonatal onset and 2 to 15 relapses over 2 to 14 years follow-up, who had complete and persistent vWF-cleaving protease deficiency (abstract; Loirat et al, Twelfth Congress of the International Pediatric Nephrology Association, Seattle, WA, USA, September 1–2, 2001) [74, 75]. Another study reported an apparent mutation in factor H (a C to T transition in SCR 20) in a family with HUS with a recessive mode of inheritance and severely depressed factor H levels [76]. However, two independent groups subsequently provided evidence that the apparent mutation was actually an artifact caused by coamplification of a factor H-related gene, and demonstrated that the real mutation in this family is an A to T transversion and a 24 bp deletion that was present in homozygosity in affected members patients and in heterozygosity in the healthy carriers (abstract; Caprioli et al, *J Am Soc Nephrol* 11:404A, 2000) [77]. Altogether, the available data provide compelling molecular evidence that genetic alterations in factor H are involved in both autosomal dominant and recessive HUS. Data that followed exposure to SLT producing *E. coli*, where only 2 to 7% of the patients progressed to overt HUS, suggest that a genetic predisposition also may have a role in the typical diarrhea-associated forms. Polymorphisms in the promoter and in the coding regions of the factor H gene have been recently described. It is intriguing to speculate that some of these polymorphic variants confer a genetic predisposition to SLT-induced HUS. Although it seems clear that familial HUS is related to an inherited congenital defect, the cause of the syndrome is probably multifactorial and the inherited complement defects simply may represent a predisposing condition that increases the risk of the disease in combination to other intercurrent environmental or acquired factors. Thus, in a family with hereditary hypocomplementemia, only one of the six members with low C3 levels developed HUS, apparently following a flu-like episode [67]. It is also possible that genetic defects in other complement regulatory proteins (DAF, CR1, CR2, C4bp) might have a role in determining low C3 in familial HUS and TTP. Data support this possibility that the above genes map on the same region of chromosome 1q as factor H [78].

CLINICAL FEATURES OF THE DIFFERENT FORMS OF THROMBOTIC MICROANGIOPATHY

Childhood HUS

Stx-associated HUS. This form, also referred to as diarrhea associated (D+) HUS, is most often due to *E. coli* O157:H7 infection and, less frequently to the O111:nonmotile, O26:HII, and O103:H_2 [reviewed in 79]. It is characterized by prodromal diarrhea followed by acute renal failure. The overall incidence is estimated to be 2.1 cases per 100,000 persons/year with a peak incidence in children younger than five years of age (6.1/100,000/year) and the lowest rate in adults 50 to 59 years old (0.5/100,000/year) [79].

E. coli O157 infection is most common in the warm summer months. The average interval between Stx-*E. coli* exposure and illness is three (range of 1 to 8) days [17]. Illness typically begins with abdominal cramps and nonbloody diarrhea; diarrhea may become hemorrhagic in 70% of cases usually within one or two days. Vomiting occurs in 30 to 60% of cases and fever in only 30%. Serum 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. Superficial ulcerations or pseudomembranes are common. *E. coli* O157 infection is complicated by HUS in 3 to 7% of the sporadic cases and up to approximately 20% or more of the epidemic forms [17]. HUS is usually diagnosed six days after the onset of diarrhea. After HUS infection, Stx-*E. coli* may be shed in the stool for several weeks after the symptoms are resolved, particularly in younger children (<5 years age) who in one-third of the cases may carry the organism for over 20 days. Use of antimotility agents and antibiotics, bloody diarrhea, fever, vomiting, elevated serum leukocyte count, extremes of age (in particular children <5 years old), and female sex have been associated with an increased risk of HUS following Stx-*E. coli* infection [17].

Fifty percent of patients who develop HUS need dialysis, 75% require red blood cell transfusions, and 25% have neurological signs, including stroke, seizure, and coma [17]. Stx-associated HUS is erroneously regarded as a benign disease. Indeed, 3 to 5% of children die in the acute phase and a similar percentage develop end-stage renal disease (ESRD) [80]. Moreover, long-term sequelae are common. In a series of 88 children, Fitzpatrick et al reported long-term renal dysfunction in 39% of cases, a residual glomerular filtration rate (GFR) <80 mL/min 173 m^2 in 18%, and persistent microalbuminuria in 31% [81]. In different series, the incidence of ESRD ranged from 3 to 18% [17]. The duration of anuria is a strong predictor of residual renal dysfunction. Indeed, only 4 of 53 children (7.5%) with a duration of anuria less than ten 10 days were found to have a residual GFR <80 mL/min as compared with 15 of 35 (42.8%) of those with anuria duration >16 days [82]. The extent of acute structural injury and loss of functioning nephron units may account for long-term sequelae of the disease. At 15 years of follow-up, Gagnadoux et al found renal sequelae in 83% of children with patchy cortical necrosis, one third of whom were in ESRD, as compared with none of those with pure glomerular TMA) [82]. Regardless of the renal damage, however, in the long term, 10 to 42% of children have some renal sequelae (such as proteinuria and/or moderate hypertension or mildly reduced GFR), 10 to 22% have chronic renal failure, and 2 to 9% ESRD) [81, 82].

Neuraminidase-associated HUS. This is a rare but potentially fatal disease that may complicate pneumonia, or less frequently, meningitis caused by *Streptococcus pneumoniae* [35, 36]. The clinical picture is usually severe, with respiratory distress, anuria, neurological involvement, and coma.

Idiopathic or atypical HUS. This is a subgroup with unknown etiology and no seasonal pattern that differs from Stx-associated HUS on epidemiologic, clinical, laboratory, and prognostic grounds. The onset is usually insidious, without antecedent diarrhea, and may be preceded by nephrotic syndrome. The disease occasionally is familial, has a tendency to relapse, and carries a worse prognosis than Stx-associated HUS [83]. Upper respiratory tract infection may trigger the disease in approximately 30% of cases [83]. The clinical syndrome resembles TTP. One-third of these patients have central nervous system involvement at onset, with convulsions and alterations of consciousness that persist despite correction of hypertension and metabolic complications of renal failure. The outcome is severe [83–85].

Adult HUS/TTP

STX-associated HUS/TTP. Albeit less frequently than in children, Stx HUS has been reported even in adults, either in epidemics involving elderly people in nursing homes or in sporadic cases [20, 33]. The etiology and pathogenesis of childhood and adult Stx HUS are the same, but renal and neurological involvement are usually more severe and sequelae more frequent in adults than in children [86]. Indeed, in older patients, a mortality rate close to 90% has been reported, a figure that is at least 10- to 20-fold higher than in pediatric series [20, 33, 86].

Acute idiopathic HUS/TTP. The defining clinical features include thrombocytopenia, microangiopathic hemolytic anemia, neurological and renal abnormalities, and fever. Neurological symptoms usually dominate the clinical picture, but usually subside within 48 hours after initiation of plasma therapy [7]. Clinical response and survival are comparable in patients with and without renal failure [5, 6].

Relapsing and frequently relapsing HUS/TTP. Relapsing forms are increasingly reported [87, 88], as more

patients than in the past recover from the initial acute episode thanks to improved supportive and specific treatments. An extremely rare variant of relapsing HUS/TTP, also known as "chronic relapsing" or "frequent relapsing" HUS/TTP, is characterized by frequent episodes, recurring after symptom-free intervals of a predictable duration (~1 month in most cases) [89].

FAMILIAL HUS/TTP

This form has a heterogeneous pattern of inheritance and presents as HUS or as TTP in several members of a given family. Less frequently, it may present as HUS in some and as TTP in other members of the same family. These are cases in which the disease had different clinical presentations in the same individual, with some episodes more reminiscent of HUS and others of TTP [67]. Decreased factor H bioavailability and abnormalities in vWF handling may have an important pathogenetic role in familial forms (see paragraph "Genetic predisposition"). Genetic counseling is important if pregnancies are planned. The outcome is usually poor with death or chronic renal failure being reported in 50 to 100% of cases [66] and post-transplant recurrences in about 50% of cases [90].

LABORATORY FINDINGS

Thrombocytopenia and microangiopathic hemolytic anemia are the laboratory hallmarks of HUS/TTP. Thrombocytopenia is usually severe, with platelet counts below 60,000/mm^3 in most cases. Platelet survival time is reduced, reflecting enhanced platelet disruption in the circulation. Giant platelets may be seen in the peripheral smear, a finding consistent with secondary activation of thrombocytopoiesis.

Anemia is usually severe, hemoglobin levels less than 10 mg/dL being reported in 99% of cases and less than 6.5 mg/dL in 40% of cases. Serum lactate dehydrogenase (LDH) levels are increased, often at very high levels, reflecting not only hemolysis, but also diffuse tissue ischemia [91]. Hyperbilirubinemia (mainly unconjugated), reticulocytosis, circulating free hemoglobin, and low or undetectable haptoglobin levels are additional aspecific indicators of the accelerated red cell disruption and production. Indeed, detection of fragmented red blood cells (schistocytes) with the typical aspect of burr or helmet cells in the peripheral smear together with a negative Coombs test (with the exception of streptococcus pneumoniae-associated HUS [35, 36]) are needed to confirm the microangiopathic nature of the hemolysis.

PATHOLOGY

The characteristic histologic lesion of HUS and TTP consists of vessel wall thickening (capillaries and arterioles), with swelling and detachment of the endothelial cells from the basement membrane and accumulation of fluffy material in the subendothelium. These changes are virtually identical and often indistinguishable from the microvascular lesions of scleroderma and malignant hypertension [2]. In HUS, the microthrombi are confined primarily to the kidneys (Fig. 2), and thus, renal failure is the dominant feature. TTP mainly involves the brain, and intravascular thrombi apparently form and disperse repeatedly, producing intermittent neurological signs. In pediatric patients, particularly in children younger than two years of age and in most cases of Stx-associated HUS, a pattern of glomerular injury is prominent (Fig. 2). Leukocyte infiltrates and thrombi are common during the early phases of the disease and usually resolve over two or three weeks. Later on, renal biopsies show ectatic glomerular capillaries, swollen endothelial cells, and some degree of necrosis. Patchy cortical necrosis may be seen in more severe cases; crescents are uncommon. Predominant arteriolar involvement, with intimal proliferation and hyperplasia, and secondary glomerular ischemia and collapse are frequently found in idiopathic and familial forms and in older children (Figs. 3 and 4) [2]. Prognosis is good in cases with predominantly glomerular changes, but is much more severe in those with primarily vascular damage (Fig. 5) or with acute cortical necrosis. Focal segmental glomerulosclerosis may be a long-term, chronic sequela of acute HUS (post-HUS chronic nephropathy) and is usually seen in children with long-lasting hypertension, proteinuria, and progressively worsening renal function [2].

Pure glomerular involvement is uncommon in adults. Vascular involvement is the predominant finding and is associated with more severe hypertension, more frequent neurological involvement, higher risk of renal and neurological sequelae, and higher mortality [92]. For instance, in 20 consecutive patients, Morel-Maroger reported only two cases with almost pure glomerular involvement who completely recovered [92]. None of the remaining 18 patients in her series recovered. Ten died, four progressed to ESRD, and four had residual chronic renal insufficiency and hypertension.

TREATMENT GUIDELINES

Childhood Stx-*E. coli*–associated HUS usually recovers spontaneously and does not require plasma therapy [7]. In contrast, a general consensus has been achieved that plasma exchange or infusion should always be tried in adult HUS/TTP to minimize the risk of death or long-term sequelae (Table 1) [7]. The outcome of secondary forms, instead, mainly depends on the prognosis of the underlying condition. Platelet count and serum LDH are the most sensitive markers for monitoring the response to plasma therapy. Treatment should be continued until complete disease remission is achieved [7]. However, no clinical parameter predicts the duration for plasma therapy. The decision to stop or continue plasma therapy is empirical. Prompt exacerbation of disease activity, principally manifested by a falling platelet count and requir-

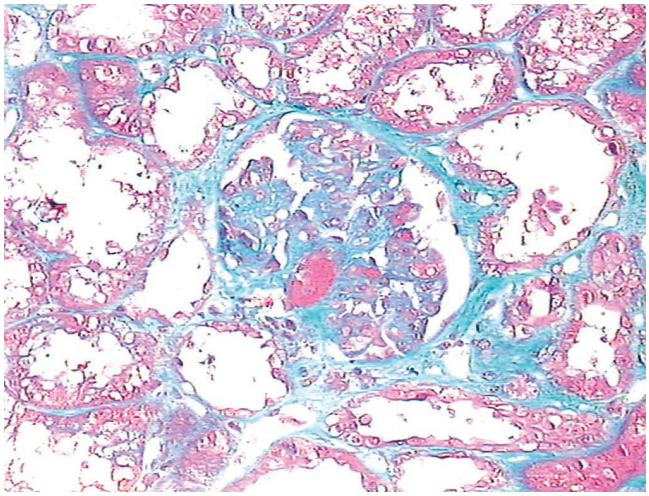

Fig. 2. Glomerulus from a patient with a form of HUS with predominant glomerular involvement. Capillary lumina are narrowed or occluded by erythrocytes and thrombi. Swelling of endothelial cells and thickening of the capillary wall with double contours have also occurred. A marked thickening of the glomerular capillary wall occurs with many double contours.

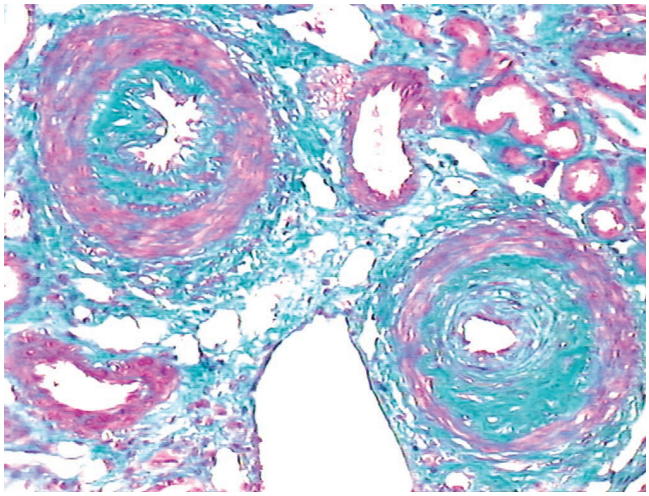

Fig. 3. Two arterioles in a case of atypical HUS with severe vascular involvement. The vascular lumen is extremely narrowed due to miointimal proliferation and vascular wall thickening.

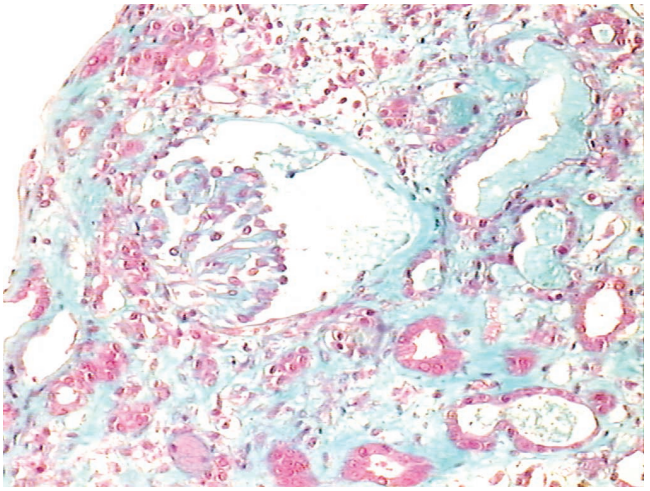

Fig. 4. Glomerulus from a patient with atypical HUS with predominant vascular involvement. Severe ischemic changes have occurred, characterized by marked restriction of the glomerular tuft.

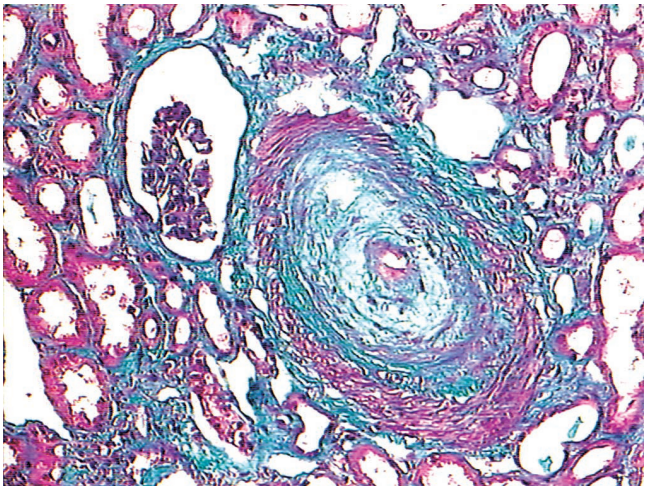

Fig. 5. A glomerulus and an interlobular artery from a patient with atypical HUS with severe vascular involvement. Severe ischemic changes have occurred with marked glomerular restriction and concomitant severe tubular changes. The vascular lumen is almost entirely occluded due to extreme thickening of the vascular wall.

ing the resumption of daily plasma therapy, is common after treatment discontinuation with reported frequencies of 29 to 82% [5, 7, 93]. Of course, this mandates additional treatment. Actually, discontinuation of plasma therapy is the only way to know whether a durable remission has been achieved, and many cycles of stopping and resuming plasma therapy may be required.

CHILDHOOD HUS

STX-associated HUS

In typical Stx-associated HUS in children, the mortality rate has significantly decreased over the last 40 years—from 40 to 50% to the current 3 to 5%—probably as the result of better supportive management of anemia, renal failure, hypertension, and electrolyte and water imbalance [79]. However, no specific therapy aimed to prevent or limit the microangiopathic process has been proven to affect the course of the disease in children. Antimotility agents may increase the risk of toxic megacolon. Antibiotics given to treat infection due to Stx–producing *E. coli* O157:H7 have been found to increase the risk of overt HUS by 17-fold [94]. Indeed, antibiotic-induced injury to the bacterial membrane might favor the acute release of large amounts of preformed toxin. Alternatively, antibiotic therapy might give *E. coli* O157:H7 a

Table 1. Specific treatments of thrombotic microangiopathy: Doses, modalities of administratioin, indications and contraindications

Treatment	Administration	Indication-comment
Treatments of proven efficacy		
Plasma exchange	1–2 plasma vol/day	First-line therapy in adult and atypical forms; life saving in cases with neurological involvement; may favor renal function recovery; no risk of fluid overload even in patients with severe cardiac or renal dysfunction
Plasma infusion	30–40 mL/kg on day 1, then 10–20 mL/kg/day	First-line therapy when exchange is not available; effective in the treatment or prevention of recurrent episodes
Plasma cryosupernatant	See plasma infusion/exchange	Second-line therapy; may be effective in cases who do not respond to whole plasma infusion/exchange
Solvent detergent-treated plasma	See plasma infusion/exchange	Same indications of plasma infusion/exchange; may limit the risk of viral contamination
Rescue treatments		
Bilateral nephrectomy		May be life saving in cases with refractory hypertension/thrombocytopenia and hypertensive encephalopathy; to be restricted to patients with biopsy evidence of severe renal vascular involvement
Splenectomy		May be effective in relapsing forms; to be restricted to patients with frequent relapses and requiring large amounts of plasma
Other treatments		
Prednisone	Oral, 60–200 mg/day, tapered by 5 mg/week	Possibly effective in mild, adult forms; additional therapy in poor responders to plasma infusion/exchange (?)
Gamma globulins	Intravenous 400 mg/kg/day	Unproven efficacy
Vincristine	1.4 mg/m^2 IV on day 1, than 1 mg every 4 days up to 4 doses	Unproven efficiacy; might decrease the risk of recurrences
Antithrombotic agents (heparin, streptokinase)	Intravenous	Unproven efficacy; increased risk of bleeding
Antiplatelet agents (aspirin, prostacyclin)	Oral or intravenous	Unproven efficacy; increased risk of bleeding
Vitamin E	Oral, 1000 mg/m^2/day	Probably effective in Stx-associated HUS; to be tested in controlled trials
Treatments under investigation		
Synsorb-PK	Oral, 0.5 g per kg body weight, $\times$7 days; to be administered at the onset of diarrhea or when ingestion of contaminated food is suspected	May prevent/limit Shiga toxin absorption and the risk of full-blown Stx-associated HUS; under clinical investigation.

selective advantage if these organisms are not as readily eliminated from the bowel as are the normal intestinal flora. Moreover, several antimicrobial drugs, particularly the quinolones, trimethoprim, and furazolidone, are potent inducers of the expression of the *Stx 2* gene and may increase the level of toxin in the intestine [95]. However, these considerations do not necessarily apply to many cases of bloody diarrhea, in particular in South America and India, which are precipitated by *E. coli* strains different from O157:H7 or by other bacteria, such as *S. dysenteriae* type 1 [96]. For instance, when hemorrhagic colitis is caused by *S. dysenteriae* type 1, early and empirical antibiotic treatment shortens the duration of diarrhea, decreases the incidence of complications, and reduces the risk of transmission by shortening the duration of bacterial shedding [97]. Thus, in developing countries where *Shigella* is the most frequent cause of hemorrhagic colitis, antibiotic therapy should be started early and even before the involved pathogen is identified [96].

New agents targeted to prevent organ exposition to Stx are currently under evaluation. The most promising are Synsorb-PK, a resin composed of repeated synthetic carbohydrate determinants linked to colloidal silica that binds Stx; recombinant modified *E. coli* that displays a Stx receptor mimic on its surface and adsorbs and neutralizes Stx with very high efficiency; and "STARFISH," an oligovalent, water-soluble carbohydrate ligand that can simultaneously engage all five B subunits of two toxin molecules. These approaches offer potent new weapons against Stx-*E. coli*–induced hemorrhagic diarrhea and HUS. Oral administration of Synsorb or recombinant modified *E. coli* could efficiently clear toxin from the gut, whereas intravenous administration of "STARFISH" might help to prevent toxin that already has entered the circulation from destroying kidney function and microvessels. Preliminary analyses of an ongoing trial in Canada found that early treatment (within two days after the onset of diarrhea) with Synsorb-PK decreased the risk of HUS from 17 to 7% [98]. Toxin-neutralizing antibodies might be a future possible prevention for *E. coli* infection. Actually, natural infection with *E. coli* O157 does not confer immunity, and no human vaccine is currently available. Nevertheless, Shiga toxoid vaccines have been shown to be effective in preventing related diseases in animals. Generalized pasteurization of ground beef through irradiation will further help to limit/prevent *E. coli* O157 and other food-borne pathogen infections.

Neuroaminidase-associated HUS. The role of plasma therapy is controversial. In theory, whole blood and plasma should be avoided, since they contain IgM and may accelerate polyagglutination and hemolysis through IgM-mediated erythrocyte and endothelial cell injury [99]. Thus, patients should be treated only with antibiotics and washed red cells [36]. In some cases, however, plasma infusion or exchange, occasionally in combination with steroids, has been associated with recovery.

Idiopathic/atypical HUS. Plasma therapy is usually recommended in these forms, although they have an almost invariably poor outcome despite the therapy.

ADULT HUS/TTP

STX-associated HUS/TTP. The evaluation of treatment efficacy in adult patients is difficult because most of the information is derived by uncontrolled series, which may include also non-Stx-HUS cases [86]. In particular, no prospective, randomized trials are available to definitely establish whether plasma infusion or exchange may offer some specific benefit as compared to supportive treatment alone. However, comparative analyses of two large series of patients treated [33] or not [20] with plasma suggest that plasma therapy may dramatically decrease overall mortality of Stx-*E. coli* O157:H7-associated HUS. These findings lead others and us to consider plasma infusion or exchange indicated in adult patients, in particular in those with severe renal insufficiency and central nervous system involvement [6, 7].

Acute idiopathic HUS/TTP. Plasma manipulation is a cornerstone in the therapy of acute idiopathic HUS/TTP. Exchange has been claimed to be superior than to plasma infusion in one study [5]. However, patients treated with exchange were given larger amounts of plasma than those treated with plasma infusion alone. Indeed, when equivalent volumes of plasma were given, infusion and exchange appeared to be equally effective [100]. Plasma exchange should be considered as first-choice therapy when renal insufficiency or heart failure limit the amount of plasma that can be provided with infusion alone. Cryosupernatant fraction (that is, plasma from which a cryoprecypitate containing the largest plasma vWF multimers, fibrinogen, and fibronectin has been removed) instead of fresh frozen plasma has been successful in treating a small number of patients who did not respond to repeated exchanges or infusions with fresh frozen plasma [101]. The rationale for this approach is that plasma cryosupernatant may provide the same beneficial factor(s) found in whole plasma, but does not contain those factors (including large vWF multimers) that may actually sustain the microangiopathic process until remission is achieved. On the basis of the previously mentioned information, the use of plasma cryosupernatant has been suggested as first line therapy of adult HUS/TTP. However, results of the few small, randomized studies failed to find differences between the two products.

Usually one plasma volume (40 mL/kg) is exchanged per session. 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 in order to minimize the recycling of infused plasma [7]. Serum LDH and platelet count

are the most reliable markers of response, and plasma therapy should be continued until they are persistently normalized. Occasional patients with idiopathic HUS/TTP do not achieve remission despite plasma therapy. Some of them become plasma-dependent and require continued treatment, since disease relapses as soon as plasma infusion or exchange is stopped. Splenectomy has been found to induce remission in some plasma-resistant cases, but was ineffective and actually increased morbidity and mortality in others [93]. Recently, the presence in the circulation of autoantibodies against vWF-cleaving protease has been claimed to help identify patients who may benefit of glucocorticoid or vincristine treatment, removal of IgG by immunoadsorption on protein A, or splenectomy [51].

Consistent data are available on the beneficial effect of bilateral nephrectomy, in particular in patients with severe renal involvement, refractory hypertension, and signs of hypertensive encephalopathy [62]. Combined to plasma therapy, antiplatelet agents have no additional effect on the outcome of the disease [5, 93]. The same considerations apply to prostacyclin, heparin, and fibrinolytic agents such as streptokinase or urokinase. Platelet transfusions are contraindicated: They may fuel the thrombotic process, and their use is recommended only in patients with active bleeding or before invasive procedures.

The use of corticosteroids has been suggested with the rationale to decrease splenic platelet sequestration and limit vascular injury. However, before plasma therapy was available, corticosteroids induced remission in fewer than 10% of patients, an outcome comparable to that of untreated patients. The same considerations apply to immunoglobulins and other immunosuppressive treatments.

RECURRENT, FAMILIAL, AND "SMOLDERING" HUS/TTP

Plasma infusion or exchange is usually effective to treat or prevent acute episodes of relapsing forms [7]. Preliminary evidence is available that elective splenectomy during hematologic remission reduces the relapse rate and the need for plasma therapy [102]. Thus, splenectomy should be considered in those patients with disabling disease requiring frequent and prolonged courses of plasma therapy. After recovery, few patients may have persistent, asymptomatic thrombocytopenia. This "smoldering" form does not need specific treatment, but warrants close monitoring since it may identify patients at risk of future recurrences [102].

SECONDARY HUS/TTP

Patients with drug-induced HUS/TTP usually promptly recover after treatment withdrawal and a short course of plasma therapy. Pregnancy-associated forms, when they manifest in the last trimester of gestation, normally recover with delivery, and plasma therapy should be restricted only to cases at imminent risk of death or with no evidence of disease recovery over 24 to 48 hours after delivery [103]. Cases occurring earlier in the course of gestation are usually episodes of acute, idiopathic HUS/TTP and are best treated with plasma therapy. Those occurring after a normal delivery are usually defined as postpartum HUS. The pathogenesis is unclear, and the outcome is usually poor with a high incidence of renal sequelae even despite aggressive plasma therapy. Cases recurring after a kidney transplant are almost invariably found in the context of a familial or recurrent background and are usually associated with a poor outcome [90]. Quite different is the outcome of de novo post-transplant HUS that may be associated with CsA or tacrolimus therapy or with vascular rejection. Removal of the drug and effective treatment of the rejection, plus plasma therapy, can cure the disease.

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