

Haemolytic-uraemic syndrome and thrombotic thrombocytopenic purpura—new insights into underlying biochemical mechanisms

Miha Furlan and Bernhard Lämmle

Central Haematology Laboratory, University Hospital, Inselspital, Bern, Switzerland

Introduction

Haemolytic-uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are two clinically similar disorders characterized by severe microangiopathic haemolytic anaemia and thrombocytopenia. HUS is characterized by thrombocytopenia, anaemia and renal insufficiency, whereas the pentad of signs and symptoms including thrombocytopenia, anaemia, neurologic deficit, renal dysfunction and fever is observed in TTP. However, about 60% of patients diagnosed with acute TTP lack one or more of these criteria, while about 30% of those receiving a diagnosis of HUS exhibit neurologic symptoms and fever. Thus, the two disorders are often difficult to distinguish. Given the lack of consistent clinical findings, the same disorder might be diagnosed as HUS by a nephrologist while a haematologist might call it TTP. Since both HUS and TTP have been reported to occur in siblings and on different occasions even in the same patient, it sometimes appears that the two disorders are different manifestations of the same pathophysiologic process. The terms TTP/HUS, microangiopathic haemolytic anaemia and thrombotic microangiopathy are sometimes used to avoid an uncertain differential diagnosis. HUS and TTP are characterized by disseminated microthrombi composed of agglutinated platelets, that occlude arterioles and capillaries in the microcirculation. In HUS the platelet microthrombi are primarily confined to the kidneys, and thus renal failure is the dominant feature. In TTP the microvascular occlusions may occur in any tissue and lead to fluctuating ischaemic dysfunction of the respective organs, most frequently of the brain, producing intermittent neurological symptoms. Markers of activated coagulation are only mildly increased in contrast to disseminated intravascular coagulation. No significant amounts of fibrin are formed and rather low amounts of fibrin degradation products are present. Blood clotting times (prothrombin time, activated partial thromboplastin time) are usually normal. The hypotheses concerning the aetiology of HUS and TTP are controversial and suggest that different pathogenetic mechanisms may trigger the disease. Acute episodes of thrombotic microangiopathies have been observed in association

with viral and bacterial infections, toxins, pregnancy, HELLP syndrome, bone marrow transplantation, drug (mitomycin, cyclosporin A, ticlopidine) therapy, and cancer, and have been variously referred to as TTP, HUS, TTP/HUS, TTP-like disease or secondary TTP. It appears that endothelial cell injury is primarily involved in the sequence of events leading to acute episodes of HUS and TTP.

Endothelial cell activation in HUS

The majority of cases with HUS occur in the childhood and are preceded by bloody diarrhoea, usually caused by some strains of enterohaemorrhagic *Escherichia coli* or *Shigella dysenteriae*. It is generally held that in diarrhoea-associated HUS (D+HUS), activation of endothelial cells with subsequent release of von Willebrand factor (vWF) and increased endothelial secretion of plasminogen activator inhibitor I (PAI-1), is central to clumping of platelets in the renal microcirculation. Shiga-toxin receptors are most abundant in the proximal tubular cells. Shiga-toxin appears to produce tubular epithelial cell damage and to induce tumour necrosis factor (TNF α) in kidneys but not in other organs [1].

Atypical HUS, also denoted as sporadic HUS, is characterized by the absence of antecedent diarrhoea (D–HUS), the tendency to relapse, a generally poor outcome, and often by a positive family history. Hypocomplementaemia and low complement C3 levels have been observed in atypical HUS. Several cases of familial HUS have been reported [2–5] with deficiency of complement factor H, also denoted as β_1 H globulin. Factor H is a cofactor for the factor I-mediated cleavage of the active form C3b of the complement factor C3. Factor H accelerates the decay of the C3 convertase and inhibits its formation. Low levels or absence of factor H result in increased activation of the alternative complement pathway. In addition to abnormal factor H, abnormalities of other regulators of the alternative pathway may also be implicated in the development of HUS.

Proteolytic cleavage of von Willebrand factor

The majority of patients who develop TTP have no identifiable associated risk factor or underlying disease.

Correspondence and offprint requests to: Miha Furlan, Central Hematology Laboratory, University Hospital, Inselspital, CH-3010 Bern, Switzerland.

TTP and HUS in adults have been associated occasionally with autoimmune phenomena (autoantibodies directed against endothelial cells or platelets, circulating immune complexes), specific platelet agglutinating plasma proteins, release of a calcium-dependent protease (calpain) or of lysosomal cathepsin-like cysteine proteases, deficiency of prostacyclin, free radical formation, and abnormal processing of vWF multimers. vWF is a plasma glycoprotein composed of a variable number of 270 kD subunits linked by disulphide bonds. vWF multimers circulating in normal human plasma range in size between 500 and 20000 kD. Unusually large vWF multimers are secreted from specific storage organelles (Weibel-Palade bodies) of endothelial cells. These highly polymeric forms of vWF may bind spontaneously to platelets at high levels of shear stress [6] and may agglutinate intact circulating platelets. Moake *et al.* [7] observed unusually large vWF multimers in plasma samples from patients with chronic relapsing forms of TTP. They tentatively predicted that in normal individuals a 'depolymerase' may be responsible for the conversion of unusually large vWF multimers to smaller polymers normally found in the circulation. This enzyme was proposed to be either a protease or a disulphide bond reductase reducing the size of the unusually large vWF multimers binding spontaneously to platelets thus giving rise to platelet thrombi within the microvasculature [7]. A specific protease that cleaves purified human vWF *in vitro* to fragments produced by *in vivo* proteolysis has been recently purified from normal plasma [8,9]. This protease is different from all known proteases, is activated by divalent cations such as Ba^{2+} and less by Ca^{2+} . Cleavage of the vWF subunit at Tyr 842-Met 843 is strongly enhanced at low ionic strength, in the presence of urea or guanidinium chloride, or under high fluid shear stress. It has been postulated that shear stress of flowing blood modulates the conformation of vWF and enhances its susceptibility to proteolysis [10].

Deficiency of von Willebrand factor-cleaving protease in TTP

An abnormal degradation of vWF has been recently established in two brothers with chronic relapsing TTP who showed unusually large vWF multimers in their plasma [11]. Both patients had complete deficiency of vWF-cleaving protease activity, whereas their parents had about half normal protease activity, and the sister had normal activity, suggesting that the deficiency of the vWF-cleaving protease may be inherited in an autosomal recessive manner. No inhibitor of the protease activity was found in the patients' plasma [11]. The recovery of vWF-cleaving protease following plasma exchange and replacement of fresh frozen plasma (FFP) in both patients with constitutional protease deficiency was about 100% and its biologic half-life was 2–4 days [12].

The lack of vWF-cleaving protease activity in

another patient with recurrent episodes of TTP was shown to be due to an acquired inhibitor of protease activity [13]. The protease inhibitor turned out to be an IgG. Prolonged treatment by plasma exchange and plasma infusion, corticosteroid and vincristine therapy resulted in disappearance of the inhibitor with transient appearance of the protease activity. In the further course of the disease, the platelet count again gradually decreased with concomitant reappearance of the inhibitor and disappearance of the vWF-cleaving protease activity leading to several relapses of acute TTP. Splenectomy, performed 1 year after the first TTP event, resulted in disappearance of the autoantibody and normalization of the protease activity, of the platelet count and haemoglobin levels.

A strong association of TTP with constitutional as well as with acquired deficiency of vWF-cleaving protease was subsequently confirmed in a retrospective multi-centre study on the prevalence of protease deficiency in patients with familial or acquired TTP [14]. Lacking or strongly decreased protease activity was found in all 30 examined patients with TTP (six familial and 24 non-familial cases) while no protease deficiency was found in 120 normal subjects. An inhibitor of vWF-cleaving protease was established in 20 of the 24 patients with non-familial TTP but in none with familial TTP. A deficiency of vWF-cleaving protease in patients with TTP was also reported by Tsai and Lian [15] who found no activity in 39 plasma samples of 37 patients obtained during the acute TTP episode. IgG antibodies with protease inhibitory activity were detected in two thirds of samples collected during the acute event. No inhibitor was found in 16 plasma samples obtained during a remission of TTP.

Normal activity of von Willebrand factor-cleaving protease in HUS

Normal protease activity was found in 21 of 23 patients with HUS (10 familial and 13 non-familial cases) [14]. Nevertheless, unusually large multimers of vWF have also been observed in plasma samples of patients with HUS [16]. We have excluded a resistance of the vWF in the plasma of these patients against proteolytic degradation by vWF-cleaving protease [14]. It is conceivable that in an acute event of HUS, the local intense stimulation or damage of renal endothelial cells leads to massive release of very large vWF multimers that escape proteolytic degradation and agglutinate platelets in the microcirculation of the kidney.

The difference between TTP and HUS permits a differential diagnosis of these two similar disorders that are often difficult to distinguish clinically. In addition, the assay of protease inhibitor may provide useful information regarding the prognosis and therapy of patients with TTP. Two new functional assays of vWF-cleaving protease have been recently described: one method is based on decreased binding of degraded vWF to collagen-coated microtiter plates [17], while the other method consists of a two-site immunoradio-

metric assay using monoclonal antibodies against the carboxyterminal and aminoterminal portion of the vWF subunit for determination of the cleaved vWF substrate [18]. The presence of an inhibitor can be detected by measuring the residual protease activity of normal plasma after incubation with patient plasma.

Management of HUS and TTP

Correction of volume depletion, electrolyte disorder and acidosis are the main therapeutic strategies in D+HUS. Control of blood pressure and early dialysis are essential in patients with renal damage. Infusion of FFP or plasma exchange have shown little if any beneficial effect in D+HUS, but the absence or presence of an unknown humoral factor that may be replaced or removed by plasma therapy, may be considered in D-HUS.

Plasma exchange and plasma infusion are considered the therapy of choice in patients with TTP. Since vWF-cleaving protease is apparently a large hydrophobic plasma protein, the question arises whether the protease activity survives treatments employed for virus inactivation of commercially available FFP. Normal activity of vWF-cleaving protease was found in FFP following virus inactivation by light in the presence of methylene blue or by the solvent/detergent procedure [14], suggesting that such virus-inactivated plasmas are appropriate for plasma exchange in patients with TTP. These observations agree with the experience of Moake *et al.* [19] who reported that solvent/detergent-treated plasma was capable of preventing relapses of thrombocytopenia in two patients with congenital chronic relapsing TTP. Repletion of vWF-cleaving protease by plasma therapy may be the only necessary treatment in patients with constitutional protease deficiency during an acute TTP episode. Prophylactic use of FFP, infused at regular intervals of 2–3 weeks, will prevent relapses in these patients. On the other hand, TTP patients with autoantibodies against vWF-cleaving protease may often need prolonged plasma exchange therapy and are sometimes resistant to plasma therapy. They may respond to additional glucocorticoid treatment, removal of IgG by immunoadsorption on protein A-Sepharose, vincristine, and splenectomy.

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