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REVIEW

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Von Willebrand factor, ADAMTS13, and thrombotic thrombocytopenic purpura

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Abstract Von Willebrand factor (vWF), a glycoprotein critical for supporting platelet adhesion and aggregation at sites of vessel injury, exists in the plasma as a series of multimers. Recent studies have shown that a metalloprotease cleaves endothelial vWF to a series of multimers. A deficiency of the protease activity due to autoimmune IgG inhibitors or genetic mutations is associated with thrombotic thrombocytopenic purpura (TTP). Positional cloning based on kindreds with a genetic deficiency of the protease and amino acid sequencing of the purified protein have identified the protease as a novel member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 repeat) zinc metalloprotease family located on the long arm of chromosome 9. Mutations of the gene are detected in patients with the congenital form of TTP. These findings support the view that vWF proteolysis is critical in regulating vWF-platelet interaction and set the stage for improving the diagnosis and treatment of thrombotic thrombocytopenic purpura.

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Abbreviations *ADAMTS:* A disintegrin and metalloprotease with thrombospondin type 1 repeat · *HUS:* Hemolytic uremic syndrome · *TTP:* Thrombotic thrombocytopenic purpura · *vWF:* Von Willlebrand factor

Introduction

Thrombotic thrombocytopenic purpura (TTP), first described by Moschcowitz [1], typically presents in previously healthy individuals with thrombocytopenia and microangiopathic hemolysis, accompanied in some cases by fever, neurological deficits, renal abnormalities, abdominal pain, elevated pancreatic enzymes, or cardiac arrhythmias. The clinical manifestations are attributable to tissue ischemia or injury resulting from thrombi in the microcirculation. Without treatment the disease is associated with a mortality rate greater than 90% [2]. When treated with plasma infusion or plasma exchange, 60–90% of the patients survive the acute episodes of TTP [3, 4]. Although TTP is an uncommon disorder, the mysterious, abrupt development of platelet-rich thrombi in the arterioles and capillaries and the dramatic response to plasma infusion or plasma exchange are intriguing. Relapses occur in more than one-third of the patients who achieve remission [5, 6]. A subset of patients develop chronic TTP and require long-term plasma exchanges.

Both endothelial injury, mediated by cytotoxic autoantibodies [7, 8] or by unknown plasma factors that promote apoptosis [9], and platelet aggregation induced by novel exogenous proteins [10] have been implicated as the underlying mechanism of thrombus formation in TTP. However, the evidence supporting these hypotheses is preliminary and does not account for all the features of TTP. Moake et al. [11] describe the presence of unusual-

ly large multimers of von Willlebrand factor (vWF) in the plasma of patients with chronic relapsing TTP, suggesting that the homeostasis of vWF is abnormal in TTP. Using electron microscopy and immunohistochemical techniques, Asada et al. [12] demonstrate that the thrombi in TTP consist of vWF and platelets. Flow cytometric analysis further reveals that the vWF on the surface of platelets is increased in the patients with TTP during the most thrombocytopenic phase [13]. These results suggest that the mechanisms preventing vWF-platelet binding in the normal circulation are disrupted in TTP.

Cell biology of von Willebrand factor

VWF, by binding to collagens and other components in the extracellular matrix and to glycoprotein receptors Ib/IX/V and $\alpha II_b\beta_3$ on the platelet surface, supports platelet adhesion, spreading, and aggregation. In in-vitro models high levels of shear stress comparable to those encountered in the arterioles and capillaries promote vWF-mediated platelet deposition at sites of vessel injury [14]. In patients with von Willebrand disease a deficiency of vWF activity results in a bleeding diathesis that may be fatal in severe cases.

VWF, synthesized as a pre-pro-vWF of 2813 amino acid residues, undergoes disulfide bonding near the carboxyl termini to form a dimer of pro-vWF in the endoplasmic reticulum [15]. As the dimers are translocated to the Golgi complex and the storage granules, the prosequence is cleaved, and the dimers are linked by disulfide bonds near the amino termini to form polymers. VWF is secreted from endothelial cells as a very large (>20,000 kDa) polymer of the mature polypeptides [16]. In the circulation a protease cleaves the endothelial vWF to a series of multimers. In some endothelial cell cultures, oligomers of pro-vWF secreted by the constitutive pathway predominate [15]. These oligomers of pro-vWF are different from the multimers observed in normal plasma: (a) pro-vWF is not present in the plasma vWF multimers; (b) the pattern of the oligomers is weighted in optical density toward smaller forms, while that of the plasma multimers is weighted toward large multimers; and (c) proteolytic fragments are present at the end of vWF multimer strands [17, 18, 19].

Cleavage of vWF by the plasma protease at the peptide bond of Tyr842-Met843 generates dimers of the 140 kDA fragment and of the 176 kDA fragment [18, 19]. Because vWF is not cleaved by the protease in vitro, the identity of the protease responsible for this cleavage and its role in hemostasis was not known. The discovery that high levels of shear stress promote proteolysis of vWF leads to the detection of the protease activity in normal plasma [19, 20]. Biochemical studies demonstrate that the protease, approximately 200 kDa in size, is similar to but distinct from the family of matrix metalloprotease [20, 21, 22].

The increase in cleavage by shear stress suggests that the conformation of vWF is flexible and responsive to shear force. Siedlecki et al. [23] using atomic force microscopy, report that the conformation of vWF is indeed responsive to shear stress: it exists in a globular conformation under static conditions, and unfolds to an elongated, filamentous form after exposure to levels of shear stress comparable to those encountered in the microcirculation. Previous studies using rotary-shadow electron microscopy also observed variation in the conformation of vWF [24]; presumably some vWF molecules underwent unfolding during the sample preparation that includes a spraying step.

Von Willebrand disease is associated with a quantitative (type 1) or qualitative (type 2) defect in vWF. In type 2A von Willebrand disease, defective vWF activity is due to a lack of large multimers. Although normal vWF is susceptible to proteolysis only after exposure to high levels of shear stress, a subset of type 2A von Willebrand factor mutants (group 2) is polymerized normally during biosynthesis but are proteolyzed by the protease under static conditions [22]. Conceivably, proteolysis of type 2A mutants occurs continuously in the circulation, while normal vWF is proteolyzed only when it is unfolded by the shear stress in the arterioles and capillaries. Because cleavage continues in the absence of shear stress, large multimers are decreased in patients with type 2A group 2 von Willebrand disease and are further decreased in vitro unless the protease activity is suppressed with EDTA [25, 26]. These findings suggest that measures that diminish the protease activity may prevent excessive cleavage of vWF and correct the bleeding diathesis in patients with type 2A von Willebrand disease.

Molecular size and the adhesive activity of vWF

Why does vWF form a large polymer, only to be cleaved to smaller forms in the circulation? One hypothesis is that the large size makes the vWF molecule flexible, enabling it to unfold under conditions of high shear stress and expose multiple binding sites for its ligands. In support of this hypothesis, large vWF multimers after a brief exposure to shear stress are more active in supporting platelet aggregation (Fig. 1). With small multimers the capacity for supporting platelet aggregation is not increased after exposure to shear stress. Similarly, shear stress also increases the binding of large vWF multimers, but not the small forms, to immobilized collagen. These findings suggest that under static conditions vWF exists in a conformation in which most binding sites are not accessible. Shear stress increases platelet aggregation or collagen binding by exposing the binding sites on vWF. The responsiveness of the adhesive capacity to shear stress provides an explanation for two unique features of vWF activity: shear stress increases vWF-supported platelet aggregation because it causes a conformational unfolding in vWF; and large multimers are more active than small forms in supporting platelet aggregation because they are more responsive to shear stress.

According to this model, unfolding by shear stress is essential for vWF to function under conditions of high shear stress. On the other hand, the unfolded forms, if allowed to accumulate, may increase the risk of vWF-platelet binding, platelet-platelet aggregation, and microvascular thrombosis (Fig. 2). Multiple platelets are brought to proximity when they are bound to the same vWF molecule, facilitating platelet-platelet interaction and aggregation. In this scheme, proteolysis of the unfolded forms of vWF by the plasma metalloprotease represents an antithrombotic mechanism that prevents vWF-platelet binding in the circulation. This model explains why a lack of this proteolytic process leads to the formation of vWF-platelet thrombi in patients with TTP [27, 28, 29, 30]. Physical entrapment may account for the restriction of the thrombi to arterioles and capillaries. Alternatively but not exclusively, the high shear stress in this part of the circulation may promote vWF unfolding and facilitate vWF-platelet binding.

The multimeric size distribution in the circulation is determined by the balance among endothelial secretion, shear stress dependent proteolytic cleavage, and con-

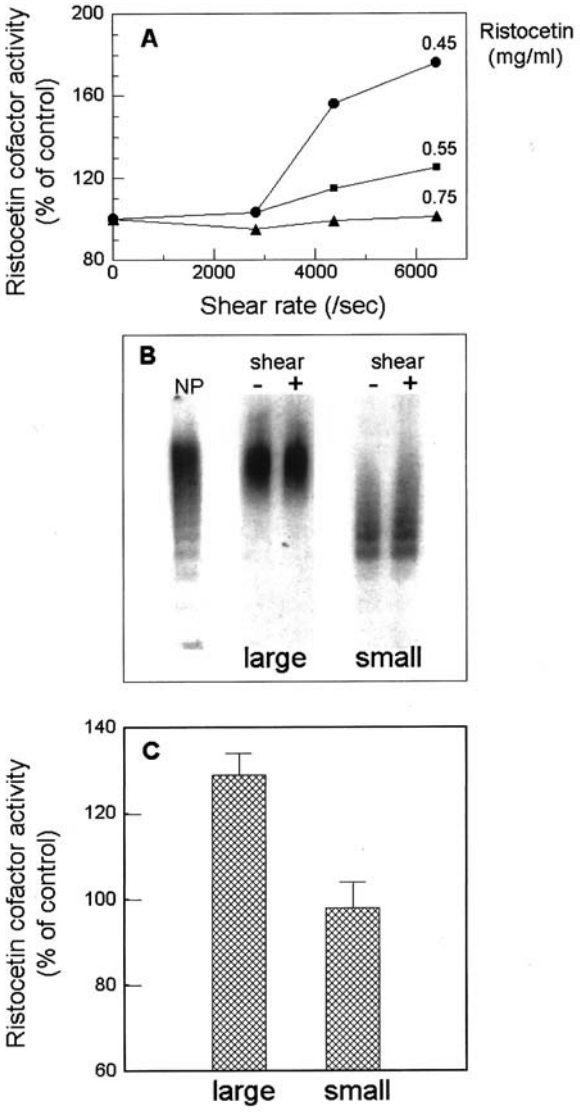

Fig. 1A–C Shear stress increases the adhesive activity of large vWF multimers. **A** vWF is exposed to shear stress in a stainless steel capillary tubing under controlled flow rates as previously described [29]. When assayed using a low ristocetin cofactor concentration (0.45 mg/ml), large vWF multimers exhibit a shear-dependent increase in ristocetin cofactor activity. **B** Large and small vWF multimers, isolated from normal plasma (*NP*), are fractionated by gel filtration and exposed to shear stress at 6404/s. After exposure to shear stress (+) the vWF size is not different from its own standing control (–). **C** Shear stress increases the ristocetin cofactor activity of the large multimers, expressed as a percentage of the standing control (mean and standard deviation) but does not affect the ristocetin cofactor activity of the small multimers. The ristocetin cofactor activity was measured using 0.45 mg/ml ristocetin

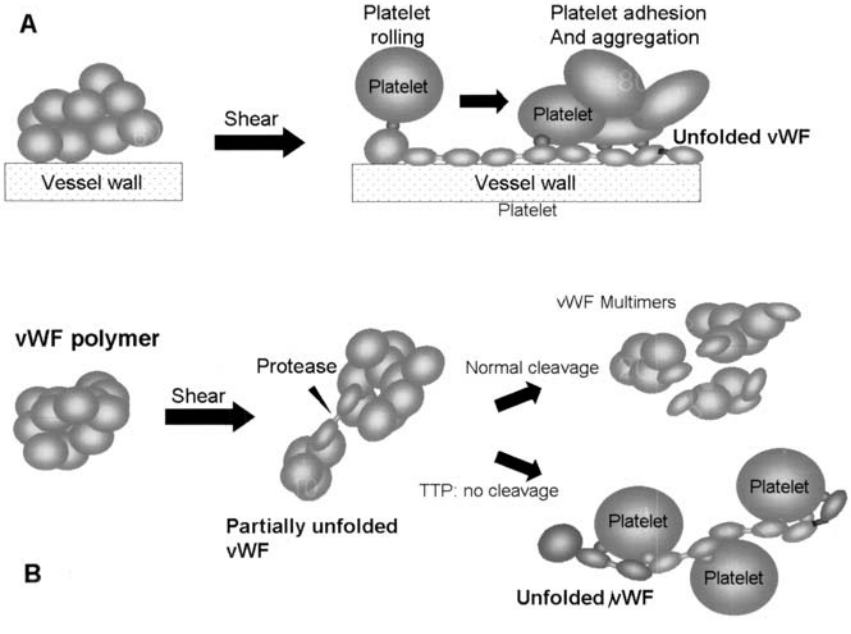

Fig. 2A, B A schematic depiction of the role of vWF proteolysis in the prevention of thrombosis. **A** The deformability allows the large vWF multimers to unfold under high shear stress, providing the substrate at sites of vessel injury for platelet rolling, adhesion, and aggregation. **B** The plasma metalloprotease cleaves unfolded forms of vWF in the circulation, generating a series of multimers. A deficiency of the protease allows unfolded forms of vWF to accumulate, resulting in vWF-platelet binding and intravascular platelet aggregation. Occlusion of the small vessels by the platelet aggregates raises the shear stress in the microcirculation, setting off a perpetuating cycle of further vWF unfolding and more platelet aggregation characteristic of TTP

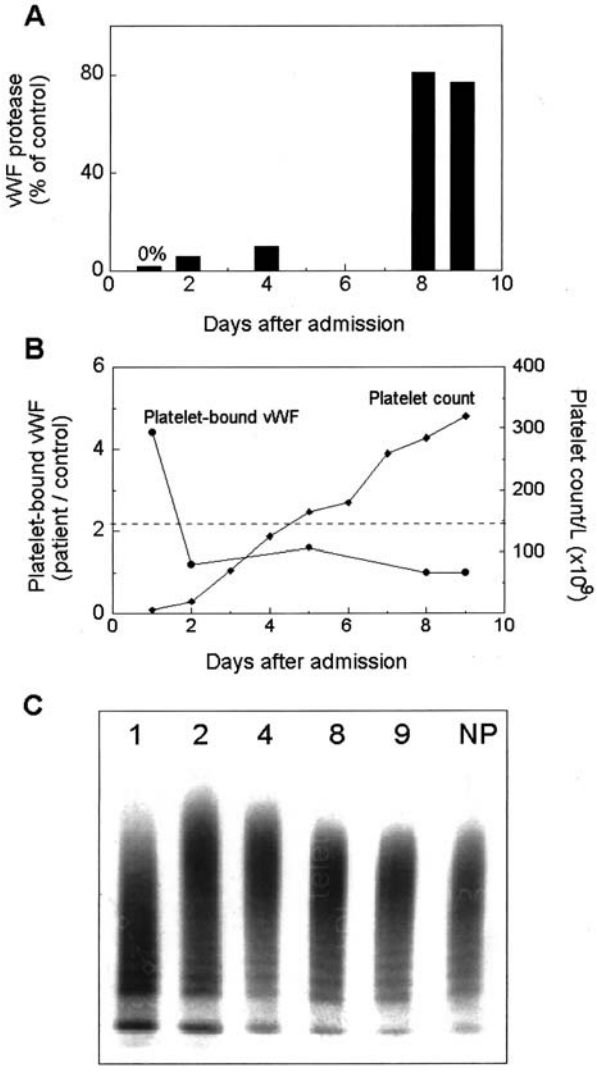

Fig. 3A–C A representative case of acute TTP depicting a biphasic change in the vWF multimer size. A lack of the vWF-cleaving metalloprotease activity at presentation (**A**, day 1) was accompanied by severe thrombocytopenia, increased platelet-bound vWF as measured by flow cytometry (**B**), and a depletion of the large multimers (**C**). After daily plasma exchange was initiated the protease level increased to approximately 10% of control by day 4 (**A**), which diminished vWF-platelet binding, increased the platelet count (**B**) and resulted in the appearance of ultralarge vWF multimers in the plasma (**C**). The protease level and the platelet count rose to the normal range on day 8 when the multimeric pattern also normalized. (The case and the flow cytometry data are the courtesy of Drs. Moake and Chow of Rice University and Baylor College of Medicine, Houston, Texas)

sumption in vWF-platelet binding. This complex process explains why vWF multimers undergo a biphasic change during the course of TTP (Fig. 3). Previously it was proposed that ultralarge multimers are more active, and that their presence predisposes patients to the development of intravascular platelet thrombosis [11]. However, further analysis of the multimer patterns in patients with TTP reveals that the process is more complex [31]. As demonstrated in Fig. 3, when a patient presents with acute TTP, the plasma metalloprotease activity is not detected, the level of platelet-bound vWF is increased, and the large multimers are decreased. In the absence of the protease activity large vWF multimers presumably unfold and bind to platelets, resulting in the increase in the platelet-bound vWF and the decrease in large multimers. Plasma exchange by raising the protease activity diminishes the vWF-platelet binding. As a result, the platelet count increases.

According to the data presented in Fig. 1 and the scheme of Fig. 2, vWF is cleaved when one or more cleavage sites are exposed during unfolding by shear stress. When the level of protease activity is decreased, cleavage of vWF is delayed, resulting in the appearance of ultralarge forms of vWF in the circulation. On the other hand, platelet binding occurs only when vWF is sufficiently unfolded by shear stress. This difference explains why, as the protease level declines, ultralarge multimers are detected before vWF-platelet binding occurs.

In patients with chronic relapsing TTP ultralarge multimers are detected because the patients are investigated before reaching the stage of severe thrombocytopenia in most instances. The subsequent course may evolve in either direction: during exacerbation of TTP vWF-platelet binding is increased; after plasma therapy a higher protease activity leads to increased cleavage of vWF. Both processes, through different mechanisms, result in a decrease in the large multimers [31, 32].

Acquired deficiency of the vWF cleaving metalloprotease

A deficiency of vWF-cleaving protease activity has been detected in patients with TTP [27, 28, 29, 30] but not in randomly selected hospitalized patients, or patients who have thrombocytopenia, hemolysis, or thrombosis from other causes [30]. IgG isolated from patients with acquired TTP inhibits the protease activity [28, 29, 30]. Thus autoimmune reaction to the protease is responsible for the protease deficiency observed in acquired TTP. In analogy to the development of cold agglutinins against the I/i antigens in some individuals following infections of mycoplasma or Epstein-Barr virus, the inhibitors of the vWF-cleaving protease may be induced by exposure to infectious agents or medications such as ticlopidine [33] and are self-limited when the inciting agents are withdrawn. In some cases the immune response persists, resulting in chronic refractory disease. Such cases may benefit from measures that decrease antibody production. In patients with intermittent relapses serial determinations of the protease activity and inhibitor titer are needed to determine whether the immune reaction persists at low levels between relapses.

The presence of inhibitors of the vWF cleaving metalloprotease in TTP explains why plasma infusion or exchange is efficacious in the treatment of TTP: plasma infusion provides the missing protease; plasma exchange is more effective because the patients are able to receive

large volume of plasma without the risk of fluid overload. Removal of the inhibitors during the exchange may also contribute to the efficacy.

However, the experience in patients with inhibitors of factor VIII suggests that replacement therapy is effective only in patients whose inhibitor titers are less than 10 U/ml. A study of the patients with TTP who participated in the randomized trial conducted by the Canadian Apheresis Group [3] showed that the inhibitor titers were indeed very low. Only 2 of the 41 cases investigated had a titer greater than 5 U/ml and none had a titer greater than 10 U/ml [34]. The reason for the low inhibitor titers has not been determined. Presumably the patients become ill from thrombotic complications soon after the protease level is suppressed to very low levels and therefore do not have sufficient time to build up high titers. Alternatively, the immunoglobulin may be directed against other antigens but exhibit cross-reactivity to the protease. Occasionally the titer of inhibitors increases during the course, leading to treatment failure and a fatal outcome [35].

Genetic deficiency of the vWF cleaving metalloprotease

A genetic deficiency of the protease causes a disorder of thrombocytopenia and microangiopathic hemolysis soon after birth. Schulman et al. [36] described the first case of what is suspected to be a congenital form of TTP in an 8-month old girl whose thrombocytopenia and microangiopathic hemolysis responded to plasma infusion. Upshaw [37] noted in a similar case that plasma infusion corrected the shortened survival time of the red blood cells and postulated that the patient lacked a plasma factor that was essential for preventing destruction of the red blood cells and platelets in the normal circulation. The presence of unusually large vWF multimers in the plasma further suggests that Schulman-Upshaw syndrome is a congenital form of TTP [38].

Ten cases with features of Schulman-Upshaw syndrome have been studied genetically [39]. These cases are characterized by the onset of disease soon after birth, either in the propositi or their siblings. Anemia and severe jaundice requiring transfusion or whole blood exchange are common immediately after birth. Ultralarge vWF multimers are present in each case. Analysis of these patients from seven pedigrees and their family members reveals that the patients have a protease level less than 10% of control; the parents are partially deficient (49–68%); and the other genetically linked members in the family are equally divided between the normal group (79–127%) and partially deficient group. The family members not genetically linked to the patients have normal protease levels, indicating that the deficiency of the protease is not caused by environmental factors.

In most genetic cases the deficiency of the vWF-cleaving metalloprotease causes a persistent disease that requires periodic plasma infusion every 2–3 weeks to prevent severe thrombocytopenia and serious complications. Other cases require plasma infusion during periods of exacerbation, which is often precipitated by stressful conditions such as fever, infection, diarrhea, surgery, and pregnancy. The variability in clinical severity indicates that disease manifestation is modified by other genetic or environmental factors.

Molecular cloning of the vWF-cleaving metalloprotease

Genomic scanning and linkage analysis performed on four kindreds localizes the defect to chromosome 9q34. Sequence analysis of genomic DNA in this region detects mutations in ADAMTS13, a novel gene of the "a disintegrin and metalloprotease with thrombospondin type 1 repeat" (ADAMTS) zinc metalloprotease family [39]. Northern blotting reveals that liver is the main organ expressing ADAMTS13. In RT-PCR the full-length mRNA and/or its alternatively spliced isoforms are expressed in the brain, placenta, ovaries, and other tissues. The functions of the isoforms are not known.

ADAMTS is a recently recognized family of zinc metalloprotease with a characteristic domain structure: a signal sequence, a propeptide sequence, a metalloprotease domain with zinc-binding motif (HxxGHxxGxxHD), a thrombospondin type 1 repeat, a cysteine-rich sequence, and a spacer sequence, which is followed by one or more thrombospondin repeats [40]. Among members of the ADAMTS family, only ADAMTS13 is known to be active in the circulation. ADAMTS13 is unique in that it has a very short propeptide sequence and a CUB domain, which may be critical for substrate recognition [41]. An RGD sequence, also found in ADAMTS2, is located after the first TSP1 repeat. ADAMTS1, ADAMTS4, and ADAMTS5 cleave proteoglycans of arterial wall (versican V1), cartilage (aggrecan) and brain (brevican). Genetically deficient mice exhibit growth retardation, fibrosis of the genitourinary system, and abnormal adipose tissues. ADAMTS4 and ADAMTS5 are believed to contribute to the destruction of cartilage in arthritis. ADAMTS2 and ADAMTS3 cleave the amino-terminal propeptides of procollagens I and II. Deficiency of ADAMTS2 causes human Ehlers-Danlos syndrome type VIIC. The functions of other members of the ADAMTS family remain unknown.

Twelve mutations have been detected in 14 of the 15 disease alleles: one splice, two frame-shift, and nine missense mutations. The missense mutations are found in the metalloprotease domain (three mutations), the thrombospondin repeats nos. 1, 3, 5, and 6 (four mutations), the cysteine-rich region (one mutation), and the CUB domain (one mutation) [39]. These domains are likely to be essential for the integrity of proteolytic activity. No recurrent mutations are detected, except in one pedigree, in which all three cases are homozygous for a single mutation. The parents of these three cases come from the same town and may have a common ancestry.

The relative paucity of obvious null mutations and the absence of patients with two copies of null mutations suggest that a complete deficiency of ADAMTS13 may be lethal, consistent with the low levels of residual VWF-cleaving protease activity observed in all ten deficient patients studied (2–9% of control).

These genetic data confirm that a deficiency of ADAMTS13 causes TTP. Three groups of investigators have purified the vWF-cleaving protease from normal plasma and mapped the partial amino acid sequence to the same ADAMTS13 gene [42, 43, 44, 45]. Together, these results establish that ADAMTS13 is the protease that cleaves vWF.

ADAMTS13 activity in hemolytic uremic syndrome and other microangiopathic disorders

Hemolytic uremic syndrome (HUS), originally described as a separate entity because it characteristically develops in young children after a bout of hemorrhagic diarrhea and has a clinical course dominated by acute renal failure, is often broadly applied to disorders that have the common features of microangiopathic hemolysis, thrombocytopenia, and renal failure. As a result the distinction between TTP and HUS becomes arbitrary. To avoid the inclusion of patients with HUS or other microangiopathic disorders our series of TTP cases exclude those patients who have plausible causes, diarrhea prodrome, prominent renal failure (maximal creatinine >353 mmol/l or requirement of dialysis), or age under 10 years. In this series, which now includes more than 120 cases, all the patients have plasma ADAMTS13 activity less than 10% of normal control. We have also studied 16 cases of typical hemolytic uremic syndrome following *Escherichia coli* O157:H7 infection and found that the ADAMTS13 activity was not decreased [46]. None of the cases in the HUS study were treated with plasma infusion or exchange, yet all recovered. As expected, the severity of renal failure was variable among the patients, and only ten required dialysis, consistent with previous observations that HUS is not always accompanied by a severe renal failure. Analysis of serial samples revealed that vWF multimer size decreases with the onset of HUS. The decrease in large multimers was associated with evidence of increased proteolysis of vWF, presumably due to abnormal shear stress in the microcirculation. Histochemical studies of the thrombi in renal glomeruli detected the presence of fibrin but not vWF. These results indicate that in HUS vWF is an innocent bystander rather than an active participant in the thrombotic process. The levels of prothrombin activation peptide F1+2 and the D-dimer are increased before the onset of HUS, further supporting the view that fibrin deposition is involved in the development of HUS [47]. Thus, idiopathic TTP and *E. coli* associated HUS are distinct in pathogenesis, although both are associated with thrombocytopenia, microangiopathic hemolysis, and renal and CNS dysfunctions. Other studies have detected low ADAMTS13 activity in some cases of HUS [28, 48, 49]. These studies may have included patients with TTP. As is discussed below, variation in the assays may also contribute to the low ADAMTS13 levels reported by these studies.

Microangiopathic hemolysis and thrombocytopenia occasionally develop in patients with metastatic cancers, bone marrow transplants, HIV infection, autoimmune collagen-vascular diseases, certain medications, disseminated intravascular coagulopathy, or pregnancy. The syndrome has been variably referred to as TTP, HUS, or TTP/HUS, without evidence that the same mechanism is involved in these disorders. Mutations in factor H gene are detected in a subset of patients with idiopathic HUS [50]. The status of ADAMTS13 and vWF proteolysis has been investigated in a small number of cases. Decreased ADAMTS13 activity due to inhibitors of the protease is detected in patients with ticlopidine use [33] but not in the patients with the syndrome of hemolysis with elevated liver enzymes and low platelet count of pregnancy [30], bone marrow transplants [51, 52], or neoplastic disorders [53]. The glomerular thrombi in the thrombotic microangiopathy following bone marrow transplantation contain fibrin and vWF, indicating that this disorder is different from TTP and *E. coli* O157:H7-associated HUS [52]. Obviously these disorders are complex and require further investigation.

Assays of the ADAMTS13 activity

Since the initial demonstration of an association between ADAMTS13 deficiency and TTP several studies have reported contradictory results. A review of the literature shows that the assays for determination of ADAMTS13 activity are different (Table 1). The difference in assays may account for some of the discrepant results reported in the literature. Since vWF in its static form is not cleaved by ADAMTS13, assay of the proteolytic activity requires a step of substrate unfolding. Shear stress is the most physiological approach to unfolding vWF. For practical reasons the assays of ADAMTS13 activity use either urea or guanidine hydrochloride. In assays that use the method originally designed by Furlan et al. [29] mixtures of vWF and test samples are dialyzed against 1.5 M urea, in which the cleavage of vWF proceeds slowly and requires an overnight incubation. It also requires the addition of barium chloride, whose potential contribution to assay variability has not been explored. Cleavage of vWF is based on a decrease in the multimer size, measured by agarose gel electrophoresis [29], a decrease in the vWF species that bind both monoclonal antibodies directed against the regions of vWF on each side of the cleavage site [54], or a decrease in collagen-binding activity [55]. Decrease in vWF multimer size may result from nonproteolytic processes such as nonspecific adsorption. Additionally, binding of vWF to collagen is unreliable because it is affected by plasma factors. In the assay used in our laboratory the vWF substrate is treated with 1.5 M guanidine hydrochloride before being added

Table 1 A comparison of ADAMTS13 assays

Method	Furlan et al. [29]	Obert et al. [54]	Gerritsen et al. [55]	Tsai et al. [46]
Substrate	vWF	vWF	vWF	vWF in 1.5 M guanidine
Reaction mixture	1.5 M urea	1.5 M urea	1.5 M urea	0.15 M guanidine
Activation	Barium chloride	Barium chloride	Barium chloride	None
Duration	Overnight	Overnight	Overnight	60 min
Detection	Decreased multimer size	Two-site immuno-radiometric assay	Decreased collagen binding	Proteolytic fragments (176-kDa dimer)
Normal range	>50%	44–178%	28–119%	79–127%

to a test sample at a dilution of 1:10 [30]. The final concentration of guanidine hydrochloride in the reaction mixtures exhibits no discernible effect on the protease activity. Since zinc cation is part of the metalloprotease, the assay does not require additional metallic cations. Cleavage of vWF occurs immediately, with the cleavage products reaching a maximum within 60 min, and proteolysis is measured by the increase in the 176-kDa dimer. A comparison of the values in normal individuals reveals that some assays produce wide normal ranges (Table 1), detecting no or very low protease levels in normal subjects and in patients without TTP [56, 57, 58]. A low ADAMTS13 level that is not associated with the presence of IgG inhibitors or ultralarge vWF multimers should raise suspicion on the validity of the assay result.

Summary and future directions

Studies on how vWF multimers are generated have led to new concepts on the regulation of vWF-platelet interaction and the identification and cloning of a novel metalloprotease ADAMTS13. Genetic mutations of ADAMTS13 result in congenital TTP, while autoimmune inhibitors of ADAMTS13 cause the acquired form of the disease. The discoveries have provided new insights into the role of vWF in the pathogenesis of TTP. The assay of ADAMTS13 activity, properly performed, is extremely useful in the management of patients with a suspected diagnosis of TTP. To translate the advances in knowledge of pathogenesis into improved diagnosis in practice, development of simple, reliable assays of ADAMTS13 that are suitable for use in clinical laboratories will be critical. Plasma exchange, used in many patients with or without TTP, is associated with potentially serious complications. Future studies should focus on strategies for more feasible ADAMTS13 replenishment and measures to suppress the production or activity of ADAMTS13 inhibitors. In this regard, the preliminary experience of rituximab, a chimeric monoclonal antibody against CD20, in patients with refractory TTP is encouraging [59]. Studies are also needed to delineate the role of plasma exchange, if any, in the management of patients without ADAMTS13 deficiency.

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