Advances in the Pathogenesis, Diagnosis, and Treatment of Thrombotic Thrombocytopenic Purpura

HAN-MOU TSAI
Division of Hematology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, New York.

Abstract. Thrombotic thrombocytopenic purpura (TTP) and the hemolytic uremic syndrome (HUS) are both characterized by thrombocytopenia, microangiopathic hemolysis, and organ dysfunction. Other disorders occasionally present with similar manifestations. Recent studies have demonstrated that deficiency in the von Willebrand factor cleaving protease ADAMTS13, due to genetic mutations or autoimmune inhibitors, causes TTP. Molecular cloning of ADAMTS13 elucidates the structure of the protease, raising the prospect for advances in diagnosis and treatment of the disease. Assay of ADAMTS13 activity distinguishes TTP from HUS and other types of thrombotic microangiopathy (TMA); therefore, the term TTP/HUS should be avoided because it obscures the known or potential differences among the various types of TMA.

Thrombotic thrombocytopenic purpura (TTP), first described in 1924, is a relatively uncommon yet intriguing disorder characterized by the mysterious, abrupt development of platelet-rich thrombi in the arterioles and capillaries and the dramatic response to plasma infusion or plasma exchange (1). For unknown reasons, the reported number of cases of TTP seems to be increasing. Although renal abnormalities are common, advanced renal failure requiring dialysis is rare. Without treatment, the disease is associated with a mortality rate of greater than 90%. When treated with plasma infusion or plasma exchange, 60% to 90% of the patients survive the acute episodes (2). Relapse occurs in more than one third of the patients who achieve remission. A subset of patients develops chronic TTP, requiring long-term plasma exchange.

The hemolytic uremic syndrome (HUS), originally described as a separate entity that characteristically develops in young children after a bout of hemorrhagic diarrhea, has a clinical course dominated by acute renal failure and occurs after infection by shiga toxin-producing microorganisms such as E. coli O157:H7 or S. dysenteriae serotype 1 (3). The severity and mortality of E. coli O157:H7–associated HUS are age-dependent. With supportive management, the mortality of E. coli–associated HUS is less than 10% among young children but may be as high as 90% among the elderly (4).

Because of its distinctive etiology and clinical features, E. coli O157:H7–associated HUS is believed to differ from TTP in pathogenesis. However, the spectrum of E. coli O157:H7–associated HUS is broader than originally recognized: it is not restricted to young children or the elderly; diarrhea may not be evident (5); the renal failure may be mild; and neurologic complications are not uncommon. These observations raised the suspicion that the HUS and TTP might have a common pathogenetic mechanism.

Further confounding the distinction, the diagnosis of HUS is also applied to disorders that have the features of microangiopathic hemolysis, thrombocytopenia, and renal failure but no evidence of infection by shiga toxin-producing microorganisms. In a subset of these patients with “atypical” HUS, mutations in factor H, which is a component of the complement regulatory proteins, are detected (6). The course of patients with factor H mutation is notable for severe hypertension and a high rate of relapse and progression to end-stage renal failure. However, at its early stage or in its mild form, these characteristic features may not be prominent. Therefore a distinction between atypical HUS, with or without factor H mutations, and TTP may not be obvious.

The syndrome of microangiopathic hemolysis and thrombocytopenia, or thrombotic microangiopathy (TMA), occasionally develops in patients with other medical conditions (Table 1). These patients have been variably referred to as TTP, HUS, or TTP/HUS, without evidence that they involve the same mechanisms. As a result, the distinction between TTP and HUS has been arbitrary. This uncertainty also leads some investigators to propose that TTP and HUS are different manifestations of a single disease entity and contributes in part to the practice of treating patients of various types of TMA with plasma exchange.

Recent studies have demonstrated that a severe deficiency of the protease that cleaves von Willebrand Factor (VWF) causes the development of TTP, suggesting that assay of the protease will distinguish TTP from HUS or other types of thrombotic microangiopathies.
von Willebrand Factor in TTP

Three previous lines of evidence suggest that VWF is involved in the pathogenesis of TTP: abnormalities in the size distribution of VWF multimers are observed in patients with TTP (7); the thrombi in TTP are enriched in VWF and platelets (8); and VWF is present on the surface of platelets in patients with TTP (9). These observations raise the possibility that VWF-platelet binding causes thrombosis in TTP.

When the presence of ultra-large VWF multimers was first described in patients with chronic relapsing TTP, it was postulated that these patients lacked a putative depolymerase or reductase that decreased the size of VWF. It was further proposed that ultra-large multimers were more likely to bind platelets, predisposing the patients to the development of platelet thrombosis in the microvasculature. However, this hypothesis does not explain why large VWF multimers normally present in the plasma also disappear when chronic TTP relapses. Similarly, in acute TTP, both large and ultra-large multimers are typically missing at the stage of severe thrombocytopenia but reappear when plasma exchange begins to increase the platelet count. Thus, while VWF may play a role in the thrombosis of TTP, the process is not restricted to ultra-large multimers.

Shear Stress, Multimer Size, and the Adhesive Activity of VWF

In experimental models, VWF binds to platelet surface glycoprotein complex Iib/IIIa through the epitopes in its A1 domain and to glycoprotein complex Iib/IIia through the RGD sequence near its carboxyl terminus. However, little binding between VWF and platelets occurs in the circulation. Lack of binding between VWF and glycoprotein complex Iib/IIia is expected because this receptor normally exists in an inactive conformation. However, there is no evidence that the glycoprotein complex Iib/IX/V requires activation. One explanation for the lack of binding between VWF and glycoprotein complex Iib/IX/V is that this interaction is actively downregulated in the circulation.

Among the adhesive proteins, VWF is unique in that high levels of shear stress promote, rather than decrease, its interaction with platelets. Furthermore, laboratory and clinical observations suggest that large multimers are hemostatically more effective than small multimers. Nevertheless, the molecular basis for the effects of shear stress and multimer size on the hemostatic function of VWF is unclear.

Biology of VWF. In ex vivo vascular preparations and some cultured endothelial cells, VWF is secreted from the Weibel-Palade bodies of endothelial cells as a very large (>20 × 10^3 kD) polymer of the mature 220-kD polypeptide (Figure 1). After its secretion, VWF undergoes proteolytic cleavage by a plasma protease in the circulation to a series of multimers. This protease, which requires calcium or zinc ion for activity, cleaves the Tyr842-Met843 bond in the central A2 domain of the VWF polypeptide, generating homodimers of the 140-kD and the 176-kD fragments (Figure 2) (10). Proteolytic cleavage explains

Table 1. Disorders associated with thrombocytopenia and microangiopathic hemolysis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Preeclampsia, HELLP syndrome</td>
</tr>
<tr>
<td>Medications</td>
<td>Cyclosporin A, tacrolimus, chemotherapeutic agents, quinine, cocaine, pentastatin, statins, ticlopidine, clopidogrel, heparin</td>
</tr>
<tr>
<td>Transplants</td>
<td>Allogeneic bone marrow, solid organs</td>
</tr>
<tr>
<td>Neoplastic diseases</td>
<td>Metastatic cancers</td>
</tr>
<tr>
<td>DIC</td>
<td>Consumption of fibrinogen and other clotting factors</td>
</tr>
<tr>
<td>Prothrombotic disorders</td>
<td>Paroxysmal nocturnal hemoglobinuria, anti-phospholipid antibody syndrome</td>
</tr>
<tr>
<td>Recent cardiovascular procedures</td>
<td>Cardiac catheterization, angioplasty, vascular bypass operations</td>
</tr>
<tr>
<td>Vascular diseases</td>
<td>Lupus, rheumatoid arthritis, scleroderma, cryoglobulinemia</td>
</tr>
<tr>
<td>Infectious diseases</td>
<td>Rocky Mountain spotted fever, anthrax</td>
</tr>
<tr>
<td>Intravascular devices</td>
<td>Prosthetic heart valves</td>
</tr>
<tr>
<td>Severe hypertension</td>
<td>&gt; 200/120 mmHg</td>
</tr>
</tbody>
</table>

Figure 1. Generation of von Willebrand factor (VWF) multimers from endothelial VWF. Endothelial cells contain a dimer of pro-VWF and a polymer of mature VWF. Only the polymer is secreted from the cells. After its secretion, the endothelial VWF is proteolyzed to a series of multimers found in the normal plasma. The various forms of VWF were separated by SDS agarose gel electrophoresis and visualized by radiolabeled antibodies of VWF. As a reference, the approximate location of IgM is indicated.
ADAMTS (adprotease, ADAMTS13, is a member of the recently recognized...

dimers of the 176-kD and the 140-kD fragments are generated. Measurement of these fragments generated from VWF substrate by SDS-PAGE and immunoblotting provides a specific assay of the activity of ADAMTS13 in the plasma (10,22).

Figure 2. A scheme depicting the steps of VWF polymerization and multimer generation. (A) Nascent VWF polypeptide consists of a prepeptide, a propeptide, and a mature polypeptide. (B) In the endoplasmic reticulum, two copies of pro-VWF are linked by disulfide bonds to form a dimer. (C) In the Weibel-Palade bodies, pro-VWF dimers are linked by disulfide bonds near the amino end to form a polymer while the propolypeptide is cleaved. (D through E) Repetitive cleavage by ADAMTS13 at the peptide bond Tyr842-Met843 converts the endothelial VWF to a series of multimers. In the process, dimers of the 176-kD and the 140-kD fragments are generated. Measurement of these fragments generated from VWF substrate by SDS-PAGE and immunoblotting provides a specific assay of the activity of ADAMTS13 in the plasma (10,22).

why two morphologically distinct units are detected at the ends of VWF multimer strands by electron microscopy (11).

Shear Stress Changes the Conformation of VWF. Although VWF and the protease that cleaves it coexist in the plasma, VWF is not further cleaved when normal plasma is incubated in vitro. Therefore, the existence of the protease remained unknown until it was found that high levels of shear stress, like those encountered in the arterioles and capillaries, enhanced the susceptibility of VWF to cleavage by the protease (10). Subsequent studies reveal that the enzyme is a zinc metalloprotease that in many aspects is similar to matrix metalloproteases (10,12–14). The protease was recently purified, and the gene encoding the protease was cloned on the basis of its partial amino acid sequence (15–18). In another study, the gene of the protease was cloned by linkage analysis on pedigrees of hereditary TTP (19). These studies revealed that this protease, ADAMTS13, is a member of the recently recognized ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 motifs) zinc metalloprotease family.

The susceptibility to cleavage by ADAMTS13 is increased by exposure to shear stress; it is therefore suspected that the conformation of VWF is flexible and responsive to shear stress. Previous studies using rotary shadow electron microscopy revealed that VWF existed in ball-of-yarn or extended filamentous forms, suggesting that its conformation was flexible (20). Studies using atomic force microscopy demonstrated that shear stress unfolded the conformation of VWF from a globular configuration to an elongated form (Figure 3) (21). It is conceivable that in its globular conformation, VWF is not accessible to ADAMTS13. Shear stress, by unfolding the conformation of VWF, exposes its cleavage sites to attack by ADAMTS13.

Other proteases, such as calpains, leukocyte elastases, cathepsin G, and plasmin, also cleave VWF. Thrombolytic therapy with streptokinase, urokinase, or tissue-type plasminogen activator in patients with myocardial infarction is associated with increased cleavage of VWF. However, under physiologic conditions, these enzymes either exist in inactive forms or are sequestered intracellularly. Physiologic inhibitors in the plasma immediately neutralize the activities of these enzymes when they are activated or released in the circulation. Furthermore, the VWF fragments produced by these enzymes are not detected in the normal plasma. Therefore except in certain pathologic conditions, these proteases are unlikely to play a role in regulating the size of VWF.

ADAMTS13 Regulates the Activity of VWF. Why is regulation of the VWF size critical? One hypothesis suggests that the function of ADAMTS13 is to regulate the adhesive activity of VWF. In the process, ADAMTS13 decreases the size of VWF multimers (Figure 4).

Responsiveness of large forms of VWF to shear stress presumably enables them to support hemostasis under high shear stress conditions. When unfolded by shear stress, VWF forms the substrate at sites of vessel injury to support platelet rolling and adhesion. This process may explain the unique capability of VWF to support platelet adhesion under conditions of high shear stress (Figure 4A). In support of this hypothesis, a brief exposure of VWF to shear stress increases its capability of supporting platelet aggregation (22,23). Furthermore the con-
formational response of VWF to shear stress is size-dependent; the large multimers, but not the small multimers, exhibit increased activity in response to shear stress (23). Thus, conformational unfolding by shear stress may explain why VWF is capable of supporting platelet adhesion under high shear stress conditions and why large multimers are hemostatically more effective than small multimers.

According to this scheme, the unfolded forms of VWF, if allowed to accumulate in the circulation, would lead to VWF-platelet binding and microvascular platelet thrombosis (Figure 4C). ADAMTS13, by cleaving the unfolded forms of VWF (Figure 4B), prevent intravascular VWF-platelet interaction. Therefore ADAMTS13 may represent a critical anti-thrombotic molecule whose function is to prevent platelet thrombosis in the circulation.

Excessive Cleavage of vWF by ADAMTS13 Causes Bleeding Diathesis. In contrast to the thrombosis associated with inadequate cleavage of VWF, excessive cleavage of VWF causes bleeding diathesis. Among the three types of von Willebrand disease, type 2 is characterized by a qualitative defect of VWF. In subtype 2A, the large multimers are decreased. In expression studies, some recombinant type 2A VWF mutants form normal large polymers. However, the VWF mutants are susceptible to ADAMTS13 under static conditions (Figure 5) (14). These observations suggest that while wild-type VWF is cleaved by ADAMTS13 only when exposed to high levels of shear stress in the arterioles and capillaries, type 2A VWF is relentlessly cleaved by ADAMTS13 throughout the circulation, causing a decrease of VWF size and bleeding diathesis (Table 2).

Deficiency of ADAMTS13

A severe decrease (<0.1 U/ml) of ADAMTS13 activity has been described in patients with TTP (19,22,24,25); autoimmune inhibitors of ADAMTS13 are detected in patients with the acquired form of TTP, while genetic mutations of ADAMTS13 are found in patients with the hereditary form of the disease (Table 2).

Inhibitors of ADAMTS13. The deficiency of ADAMTS13 activity in patients with sporadic TTP appears to be autoimmune in nature. IgG molecules isolated from patients with TTP suppress the ADAMTS13 activity in normal plasma (22,24,25). The inhibition is reversed by pre-incubation of the IgG molecules with anti-Fab but not with anti-Fc, indicating that the inhibition involves antigen-antibody interaction (22). Inhibitors of ADAMTS13 are detected in 70 to 80% of the cases with sporadic TTP. Among the remaining cases, the levels of inhibitors may be below the limit of detection by the conventional mixing methods. In support of this explanation, when the inhibitor-negative samples are re-investigated by using a higher patient to control plasma volume ratio or by

Figure 4. Scheme depicting the role of shear stress and ADAMTS13 in regulating the interaction between VWF and platelets. (A) A large VWF multimer is unfolded by shear stress on an injured vessel wall, forming the substrate for supporting platelet rolling, adhesion, and aggregation, a critical step for hemostasis under the high shear stress conditions in the capillaries and arterioles. (B) In the circulation, ADAMTS13 cleaves VWF whenever its cleavage sites are exposed by shear stress. This process prevents the VWF from becoming fully unfolded. In the process, smaller multimers are generated. (C) In the absence of ADAMTS13, VWF becomes unfolded, causing the formation of platelet thrombi in the arterioles and capillaries. Deficiency of ADAMTS13 results in a decrease in the cleavage of VWF and the appearance of ultra-large multimers. The conformation of ultra-large and large VWF multimers is responsive to shear stress; therefore, the process of VWF-platelet binding in the absence of ADAMTS13 provides an explanation of why both large and ultra-large forms of VWF are depleted in thrombotic thrombocytopenic purpura (TTP) when thrombocytopenia is severe (<20 to 30 × 10^9/L).

Figure 5. Schematic depiction of the mutations of the VWF gene that cause excessive cleavage of VWF by ADAMTS13 and bleeding diathesis. Some mutations in the A2 domain of VWF, where the cleavage site (Tyr842-Met843) is located, increase the susceptibility of VWF to cleavage by ADAMTS13. Because of increased cleavage by ADAMTS13, the large multimers are characteristically decreased in type 2A von Willebrand disease. Conversely, mutations that decrease the cleavability of VWF by ADAMTS13 may cause thrombosis similar to TTP. However, this hypothetical disease would not respond to plasma infusion. The ligands that bind the epitopes in the A1 and the A3 domains are indicated. ◊, N-linked CHO; ●, O-linked CHO; ○, cysteine residues.
using IgG molecules isolated from the patients, an inhibitory activity is detected.

Most patients of TTP do not have obvious causes. Speculatively, infectious agents may induce autoimmune reaction to ADAMTS13. In analogy to the development of cold agglutinins against the red blood cell I/i antigens in some individuals after infections of mycoplasma or Epstein-Barr virus, certain infections that are innocuous in most people may induce in susceptible individuals the production of inhibitors of ADAMTS13. A subsequent decrease in the titer of ADAMTS13 inhibitors allows the patients to remain in remission after they survive the acute episode. In patients who are treated with ticlopidine for intravascular stents or strokes, inhibitors of ADAMTS13 occasionally develop a few weeks after initiation of the therapy and disappear after the medication is discontinued (26).

**Hereditary Deficiency of ADAMTS13.** Schulman-Upshaw syndrome is characterized by the development of thrombocytopenia and microangiopathic hemolysis soon after birth that is alleviated after plasma infusion. During the early neonatal period, the patients are often treated with blood transfusion for anemia or whole blood exchange transfusion for severe jaundice. Either treatment is followed by clinical improvement. While most patients subsequently require plasma infusion every 2 to 4 wk, a subset of patients develops relapse of thrombocytopenia and microangiopathic hemolysis intermittently, often but not always triggered by fever, infection, diarrhea, surgery, or pregnancy. Renal dysfunction and neurologic complications may occur during episodes of severe exacerbation. Although renal dysfunction is occasionally severe, it typically resolves after plasma therapy.

Furlan et al. (27) report a deficiency of VWF cleaving protease in four patients with chronic relapsing TTP. Ten cases with features of the Schulman-Upshaw syndrome have been studied genetically (19). The protease level is less than 0.1 U/ml in all the patients and partially decreased (0.49 to 0.68 U/ml) in the parents. The other genetically linked members in the families either have normal (0.79 to 1.27 U/ml) or partially deficient protease activity levels. The family members not genetically linked to the patients have normal protease levels, excluding the possibility that environmental factors cause ADAMTS13 deficiency. Severe deficiency in ADAMTS13 is corroborated in each case by the presence of ultra-large VWF multimers in the plasma (19).

**Molecular Cloning of ADAMTS13**

The identification of carriers among the family members of patients with Schulman-Upshaw syndrome increases the genetic power than would be available from analysis of clinical phenotype alone. A genome-wide scan performed on the first four pedigrees with 382 microsatellite markers links the deficiency in VWF cleavage to the ADAMTS13 gene on chromosome 9 (q34) (19). The deduced protein sequence of ADAMTS13, a member of the recently recognized ADAMTS zinc metalloprotease family, contains 1427 amino acid residues (Figure 6). Other investigators have cloned the same gene based on the partial amino acid sequence of the purified protease.

**Table 2.** Hemostatic defects caused by abnormal von Willebrand factor (VWF) proteolysis

<table>
<thead>
<tr>
<th>Disease Type 2A (group 2) von Willebrand Disease</th>
<th>Thrombotic Thrombocytopenic Purpura (TTP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cause</strong></td>
<td>Genetic mutations of VWF</td>
</tr>
<tr>
<td><strong>Defect</strong></td>
<td>Excessive cleavage of VWF</td>
</tr>
<tr>
<td><strong>VWF phenotype</strong></td>
<td>Decreased large multimers</td>
</tr>
<tr>
<td><strong>Manifestation</strong></td>
<td>Bleeding diathesis</td>
</tr>
<tr>
<td><strong>Therapy</strong></td>
<td>Desmopressin</td>
</tr>
<tr>
<td></td>
<td>VWF replacement</td>
</tr>
</tbody>
</table>

**Figure 6.** Domain structure of VWF cleaving protease ADAMTS13. The protease belongs to the recently recognized zinc metalloprotease ADAMTS (a disintegrin and metalloprotease with thrombospondin type 1 repeats) family with the common domain structure of a signal peptide, a propeptide that is cleaved at the end of a RXRR sequence, a type M12B metalloprotease domain with a zinc-binding motif (HExGHxxGxxHD) and disintegrin-like sequence, a cystein-rich region, a spacer domain, and one or more thrombospondin type 1 repeats. Among the ADAMTS proteases, ADAMTS13 uniquely contains CUB domains that may be involved in substrate recognition. The approximate locations of missense (▲), frameshift or splice (■) and nonsense mutations (×) described in three genetic studies are depicted.
Clinical Implications

The mature ADAMTS13 has a calculated polypeptide mass of 145 kD, compared with the apparent mass of 190 kD for the protease purified from human plasma. The difference is probably due to glycosylation; ADAMTS13 has 10 potential N-glycosylation sites, and the TSP1 repeats have additional sites for other oligosaccharides (18).

Alternative splicing generates at least four transcript variants encoding different isoforms of ADAMTS13 that have identical N- and C-termini (17–19). These studies also demonstrated that the full-length 4.7-kb mRNA is expressed mainly in the liver. However, RT-PCR showed that the protease or its isoforms are expressed in other tissues.

In situ hybridization studies suggest that ADAMTS13 is synthesized in perisinusoidal cells of the liver but not in the hepatocytes (28). This observation may explain why ADAMTS13 is expressed in multiple tissues and why in advanced liver diseases, the activity level of ADAMTS13 is normal or mildly decreased (28), instead of markedly decreased as previously reported (29).

Mutations of ADAMTS13. Twelve mutations of ADAMTS13 were initially detected in 14 of the 15 disease alleles: one splice, two frame-shift, and nine missense mutations (Figure 6). The missense mutations are found in the metallocproteinase domain, the central thrombospondin type 1 motif, the cystein-rich region (one mutation in each), and other less-conserved regions (19). Subsequent studies have reported additional mutations (30,31). Expression studies reveal that the mutations abrogate the protease activity or impair its biosynthesis (30,32). These data suggest that multiple, physically separated regions of ADAMTS13 are involved in the expression of its enzymatic activity.

In the first genetically investigated series of hereditary TTP, different mutations were detected, except in one pedigree, in which all three cases were homozygous for a single mutation (19). The parents of these three cases, originally from the same geographical region, might have a common ancestry. The presence of distinct mutations among the pedigrees and of multiple polymorphic sites in the population (19) suggests that ADAMTS13 gene is prone to mutation. One mutation (P475S) appears to a polymorphic variant in the Japanese population (30). These observations suggest that genetic deficiency of ADAMTS13 may be more prevalent in certain populations.

Clinical Implications

Genetic Deficiency of ADAMTS13. Because the disease may remain quiescent after the neonatal period, hereditary TTP should not be dismissed because of a late onset or a lack of family history. When inhibitors are not detected in a patient, the parents and other family members should be investigated and genetic studies performed to detect mutations of the ADAMTS13 gene. Identification of a genetic cause has important implications for counseling and treatment. Patients with hereditary TTP do not have inhibitors of the ADAMTS13 and typically respond to infusion of 10 to 15 ml/kg plasma, because a very low level of ADAMTS13 activity (0.15 to 0.2 U/ml) is sufficient for inducing remission of thrombocytopenia and microangiopathic hemolysis. Interestingly, platelet transfusion also results in clinical improvement (33), while it often causes exacerbation in acquired TTP. In the absence of inhibitors, the small amount of plasma in the platelet concentrates is presumably sufficient for raising ADAMTS13 activity to a therapeutic level in the patients with hereditary TTP. A small amount of plasma infusion is associated with remission; therefore, plasma exchange, which is more expensive and has risk of additional complications, is unnecessary in patients with genetic deficiency of ADAMTS13. Similarly, splenectomy or other modalities of therapies to suppress the immune response are unlikely to benefit the patients.

Inhibitors of ADAMTS13. Patients with the acquired form of TTP typically have an ADAMTS13 activity level less than 0.1 U/ml when they present with thrombocytopenia and microangiopathic hemolysis. When the inhibitors are not detected by plasma mixing studies, IgG molecules should be isolated from the patient for determination of inhibitory activity against ADAMTS13.

Because of the presence of inhibitors, treatment of acquired TTP requires a large amount of plasma. Plasma exchange is more effective than plasma infusion, presumably because it allows the patients to receive large amount of plasma to overcome the inhibitors without imposing the risk of fluid overload. Removal of the inhibitors during plasma exchange may also contribute to its efficacy. Occasionally, the titer of inhibitors increases during the course of plasma exchange, resulting in treatment failure and a fatal outcome (34). In the future, measures that effectively remove the inhibitors or prevent the rise of inhibitor titers during therapy may decrease the risk of treatment failure and death.

Low levels of inhibitors may cause a persistent decrease of the ADAMTS13 activity level for months to years without apparent complications. However, a subset of patients has frequent relapses or a refractory disease that requires long-term plasma exchange. In such patients, anti-platelet agents, vincristine, splenectomy, cyclophosphamide, azathioprine, Staphylococcal protein A columns, or high-dose immunoglobulins has been used empirically with variable success. Recently, rituximab, a chimeric monoclonal anti-CD20 that depletes B cells from the circulation and the tissues, has shown promising results of decreasing the inhibitor titers and inducing long-term remission in patients with refractory TTP or recurrent TIA (35,36). Further investigation is needed to delineate the efficacy of these therapies.

VWF and ADAMTS13 Activity Levels in Other Types of Thrombotic Microangiopathy

The pathogenesis of TTP was unknown before ADAMTS13 was identified; therefore, no clinical criteria existed to reliably distinguish TTP from HUS or other types of TMA. In assigning patients presenting with thrombocytopenia and microangiopathic hemolysis to either TTP or HUS, as some studies did (25,37–39), uncertainty of diagnosis is a concern.

To identify a group of patients that is likely to have a common pathogenesis, we include in the TTP series only the cases without apparent complications, is unnecessary in patients with genetic deficiency of ADAMTS13. Similarly, splenectomy or other modalities of therapies to suppress the immune response are unlikely to benefit the patients.
Table 3. Criteria for inclusion of TTP cases

<table>
<thead>
<tr>
<th>Presence of thrombocytopenia and microangiopathic hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No plausible causes (Table 1)</td>
</tr>
<tr>
<td>No features suggestive of typical or atypical HUS</td>
</tr>
<tr>
<td>preceding diarrhea</td>
</tr>
<tr>
<td>oliguria or anuria</td>
</tr>
<tr>
<td>hypertension (&gt;140/90 mmHg)</td>
</tr>
<tr>
<td>creatinine &gt; 3.5 mg/dL</td>
</tr>
<tr>
<td>requirement of dialysis therapy</td>
</tr>
<tr>
<td>pulmonary infiltrates or respiratory distress</td>
</tr>
<tr>
<td>Age greater than 10 yr</td>
</tr>
</tbody>
</table>

Several studies did not provide diagnostic criteria; instead they relied on the referring clinicians for diagnosis (25,37,39). In one study, HUS included patients with evidence of an increase in the creatinine concentration, while TTP included patients with evidence of neurologic complications (38). This case definition is problematic for three reasons: first, TTP and HUS are two examples of a group of disorders that are associated with microangiopathic hemolysis and thrombocytopenia occasion-
ally develop in other disorders (Table 1). Preliminary data from a limited number of cases suggest that the pathogenesis among these patients is heterogeneous. Among the patients who develop thrombocytopenia and microangiopathic hemolysis after the initiation of ticlopidine therapy, the ADAMTS13 activity is profoundly decreased and IgG inhibitors of the protease are detected (26). Ticlopidine may induce an autoimmune reaction to ADAMTS13 via a mechanism somewhat analogous to that in α-methyl dopa–induced autoimmune hemolytic reaction. No inhibitors of ADAMTS13 are detected in patients with HELLP syndrome (22), allogeneic bone marrow transplants (43,44), or neoplastic disorders (28,45). One study reported that VWF cleaving activity was very low in patients with metastatic neoplasms (46); however, the accuracy of the assay used in that study was not well characterized. In bone marrow transplant–associated TMA, the glomerular thrombi contain fibrin and VWF, suggesting that this disorder is different from both TTP and E. coli O157:H7–associated HUS (44).

More studies are needed to determine how common ADAMTS13 deficiency plays a role in patients with plausible causes of TMA. Nevertheless, the present data indicates that TMA is a histopathologic condition common to many disorders of diverse pathogenetic mechanisms (Figure 7). The term TTP/HUS should be avoided because it obscures the known or potential differences among the various types of TMA.

Specificity of ADAMTS13 Deficiency

Several studies have raised question on the association between TTP and severe ADAMTS13 deficiency (29,38,47). Difference in case definition and assay design probably contribute to the discrepancy.

Some studies did not provide diagnostic criteria; instead they relied on the referring clinicians for diagnosis (25,37,39). In one study, HUS included patients with evidence of an increase in the creatinine concentration, while TTP included patients with evidence of neurologic complications (38). This case definition is problematic for three reasons: first, TTP and HUS...
do not encompass all cases with thrombocytopenia and microangiopathic hemolysis; second, there is no basis to exclude TTP because of a mild increase of the creatinine concentration; third, neurologic complications occasionally occur in patients with E. coli–associated HUS or factor H deficiency and are not always present in patients with TTP.

Variation in the methods of measuring ADAMTS13 activity is also critical. Current assays differ in experimental designs and produce different ranges of values among normal individuals (Table 4). The difference may explain why our assay detects normal or slightly decreased ADAMTS13 levels in the umbilical cord blood and among patients with metastatic cancers, liver diseases, heparin-induced thrombocytopenia, or thrombocytopenia of any causes (22,28,48), while some studies report very low ADAMTS13 values among patients with similar conditions, normal individuals, or neonates (29,39,46). The causes of low values in an assay may differ from the causes of high values; therefore, a bias toward low values would occur if the causes of low values (e.g., high VWF antigen levels in assays based on multimer size change) were accentuated among patients with pathologic conditions.

Because of the difference in case definition and assay methods, results reported from different laboratories are not directly comparable. Importantly, assay of VWF cleaving activity is a complex procedure that involves the use of nonphysiologic conditions; therefore, the significance of a low ADAMTS13 value should be interpreted with caution in the absence of corroborative evidences (e.g., decreased cleavage of VWF by ADAMTS13, the presence of inhibitors, or genetic mutations). Although ADAMTS13 is stable for at least 20 yr in frozen normal plasma (22), its stability in pathologic samples has not been systemically investigated. Furthermore, the possibility that certain factors in pathologic conditions cause a bias toward lower values has not been excluded. Until the possibility of artifacts is addressed, a low ADAMTS13 assay result that is not accompanied by evidence of a biologic cause or consequence of ADAMTS13 deficiency should not equate unequivocally with a decreased level found in the patient.

As an example to demonstrate the difference among the assays, one report previously described the presence of normal VWF cleaving activity in a patient with characteristics of hereditary TTP: thrombocytopenia and microangiopathic hemolysis since early infancy that were alleviated by periodic plasma infusion and the presence of ultra-large VWF multimers (49). Re-analysis of this patient and her parents by using our assay revealed that the patients had a very low ADAMTS13 activity level and that both parents had partially decreased levels. The deficiency in this family was confirmed by DNA sequence analysis of the ADAMTS13 gene, which detected a homozygous dinucleotide deletion in the patient (50). The experience in this case highlights the critical need for prudence when an ADAMTS13 assay result does not correlate with the expected change of VWF multimers.

Definition of TTP and Future Directions

Studies on the mechanism of VWF multimer generation have led to the identification and cloning of a novel metalloprotease, ADAMTS13, which is essential for preventing the development of platelet thrombi in the normal circulation. The data reviewed here suggests that TTP may be defined as a pro-thrombotic state in the microvasculature caused by severe ADAMTS13 deficiency (Table 5). This definition identifies a group of patients who have a common pathogenetic mechanism and may potentially benefit from replacement with ADAMTS13 protein or ADAMTS13 gene therapy in the future.

Table 5. A definition of TTP based on ADAMTS13 deficiency

<table>
<thead>
<tr>
<th>Low ADAMTS13 activity</th>
<th>Evidence of biological effects of low ADAMTS13 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Causes</td>
<td>• decreased proteolysis of VWF (ultra-large multimers)</td>
</tr>
<tr>
<td></td>
<td>• genetic mutations of ADAMTS13</td>
</tr>
<tr>
<td></td>
<td>• inhibitors of ADAMTS13</td>
</tr>
<tr>
<td></td>
<td>• others?</td>
</tr>
<tr>
<td>Propensity to microvascular thrombosis, presenting with:</td>
<td>• VWF-rich thrombi in arterioles and capillaries</td>
</tr>
<tr>
<td></td>
<td>• thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td>• microangiopathic hemolysis</td>
</tr>
<tr>
<td></td>
<td>• dysfunction of organs</td>
</tr>
</tbody>
</table>

Table 4. A comparison of ADAMTS13 assays

<table>
<thead>
<tr>
<th>Type of Assay</th>
<th>Furlan, et al. (24)</th>
<th>Tsai, et al. (22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate</td>
<td>VWF</td>
<td>VWF in 1.5 M guanidine</td>
</tr>
<tr>
<td>Reaction mixture</td>
<td>1.5 M urea</td>
<td>0.15 M guanidine None</td>
</tr>
<tr>
<td>Activation</td>
<td>Barium chloride</td>
<td>None</td>
</tr>
<tr>
<td>Incubation</td>
<td>Overnight</td>
<td>60 min</td>
</tr>
<tr>
<td>Detection</td>
<td>Decreased multimer size</td>
<td>Two-site immunoradiometric assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased collagen binding activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteolytic fragments (dimer of the 176-kD fragment)</td>
</tr>
<tr>
<td>Normal range</td>
<td>&gt;50% (25)</td>
<td>44% to 200% (37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40% to 170% (29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79% to 127% (40)</td>
</tr>
</tbody>
</table>

*This table depicts the differences among the representative assays in experimental designs and normal ranges. The difference is one reason why results reported from different laboratories are not directly comparable.
To avoid incorrect diagnosis due to problems of ADAMTS13 assays, a low protease value should be corroborated by evidence of decreased VWF proteolysis and the presence of inhibitors or deficiency in the parents or other family members. Speculatively, defective biosynthesis, increased clearance, decreased stability, or non-autoimmune inhibitors may decrease the level of ADAMTS13 activity. However, the potential role of these mechanisms in decreasing ADAMTS13 activity levels has not been demonstrated.

This definition does not require the presence of TMA at the time of diagnosis. The definition helps identify patients who are predisposed to developing thrombotic complications in the absence of characteristic hemolytic changes commonly expected of TTP and is particularly useful in patients with multiple medical conditions whose cause of TMA is otherwise uncertain. In a patient with recurrent TIA due to TTP, ADAMTS13 analysis led to the correct diagnosis in the absence of thrombocytopenia or microangiopathic hemolysis (36). Inhibitors caused the deficiency of ADAMTS13; therefore, she was treated with rituximab, which led to decreased inhibitor titer, increased ADAMTS13 activity level, and resolution of her TIA.

Conversely, in a patient presenting with TMA, the presence of normal ADAMTS13 activity should prompt searches for other causes. In our experience, this strategy has led to the diagnosis of occult metastatic cancers and paroxysmal nocturnal hemoglobinuria. This definition also highlights the importance of developing easy, reliable assays for use in clinical laboratories, a hitherto elusive goal.

Plasma infusion or exchange, which is expensive and occasionally associated with potentially serious complications, is often used in patients without ADAMTS13 deficiency. TMA is a syndrome caused by multiple disorders; therefore, future studies should also focus on delineating the role of plasma therapy in the management of patients without ADAMTS13 deficiency.

Acknowledgments
This work was supported in part by a grant (R01 HL 62136) from the National Heart, Lung and Blood Institute of the NIH.

References


28. Lee TP, Bouhassira EE, Lyubsky S, Tsai HM: ADAMTS13, the von Willebrand factor cleaving metalloprotease, is expressed in the perisinusoidal cells of the liver. *Blood* 100[Suppl 1]: 497a, 2002


41. Chow TW, Hellums JD, Moake JL: Erythrocyte fragments can be produced in vitro by high shear stress and quantified by flow cytometry in thrombotic thrombocytopenic purpura (TTP) blood. *Blood* 94[Suppl 1]: 452a, 1999


