Deficiency of ADAMTS13 Causes Thrombotic Thrombocytopenic Purpura
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Abstract—In the circulation, a plasma metalloprotease, ADAMTS13, cleaves von Willebrand factor (vWF) in a shear-dependent manner. This article reviews the role of this cleavage in regulating vWF-platelet interaction and proposes a scheme for understanding how a deficiency of ADAMTS13 results in the development of microthrombi in patients with thrombotic thrombocytopenic purpura. (Arterioscler Thromb Vasc Biol. 2003;23:388-396.)

Key Words: von Willebrand factor ■ thrombotic thrombocytopenic purpura ■ ADAMTS13 ■ shear stress ■ von Willebrand factor cleaving protease

Von Willebrand factor (vWF), a multimeric glycoprotein synthesized in endothelial cells and megakaryocytes, supports platelet adhesion and aggregation in conditions of high shear stress like those in the arterioles and capillaries. A deficiency in vWF causes bleeding diathesis in patients with von Willebrand disease. Two platelet-surface glycoprotein complexes, Ib/IX/V and $\alpha_\text{IIb}\beta_3$, bind vWF. vWF-platelet binding is minimal in the normal circulation. A lack of interaction between vWF and glycoprotein $\alpha_\text{IIb}\beta_3$ is expected because this receptor normally exists in an inactive form. In contrast, there is no evidence that glycoprotein Ib/IX/V requires activation. One explanation of this lack of binding is that the vWF-platelet interaction is actively downregulated in the circulation.

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This article reviews the putative role of vWF proteolysis by ADAMTS (A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats) 13 in preventing vWF-platelet binding and how a deficiency of this protease is linked to the development of microvascular thrombosis characteristic of thrombotic thrombocytopenic purpura (TTP), a hitherto mysterious disease.

ADAMTS13 Regulates the Size of vWF
Studies with cultured endothelial cells and ex vivo vascular preparations reveal that vWF is secreted from endothelial cells by the regulated pathway as a very large (>$2\times10^3$ kDa) polymer of the mature 220-kDa polypeptide (Figure 1A through 1C).1,2 After its secretion from endothelial cells, vWF is cleaved by ADAMTS13 in the circulation to a series of multimers (Figure 1D and 1E). This protease, which requires calcium or zinc ion for activity, cleaves the Tyr842-Met843 bond in the central A2 domain of the mature vWF polypeptide.3 Cleavage of vWF explains why 2 morphologically distinct types of ends are present in vWF multimer strands.4 Although vWF and ADAMTS13 coexist in the plasma, incubation of vWF with the protease does not lead to its cleavage. As a result, the identity of the protease was unknown until it was discovered that vWF is susceptible to the protease only after exposure to high levels of shear stress, like those encountered in the arterioles and capillaries, or to chaotropic agents, such as urea or guanidine hydrochloride.5–7 These findings led to the identification and cloning of the protease.

Studies in animal models provide direct evidence that ADAMTS13 is critical in determining the size of vWF. Infusion of partially purified ADAMTS13 into mice causes a decrease in the size of vWF (Figure 2A). Conversely, infusion of inhibitory IgG molecules obtained from a patient with TTP suppressed the protease activity and increased the size of vWF (Figure 2B). These changes demonstrate that ADAMTS13 regulates the size of vWF in the circulation.
ADAMTS13 and Type 2A von Willebrand Disease

Among the 3 types of von Willebrand disease, type 2 is characterized by a qualitative defect of vWF. In subtype 2A, the large multimers are decreased. However, in expression studies, a subset of the type 2A vWF mutants (group 2) are secreted as normal large polymers, suggesting that the lack of large multimers in patients with type 2A, group 2 von Willebrand disease is not caused by defects in biosynthesis; instead, it occurs after secretion. Incubation of the type 2A vWF mutants with normal plasma or partially purified ADAMTS13 reveals that the type 2A vWF mutants are cleaved by ADAMTS13 under static conditions. It is conceivable that in normal individuals, vWF is cleaved by ADAMTS13 only when it is exposed to high levels of shear stress in the arterioles or capillaries, whereas in patients with type 2A, group 2 von Willebrand disease, the mutated vWF is cleaved relentlessly throughout the circulation, resulting in a decrease in the size of the multimers. The size of vWF further decreases in vitro, unless the protease activity in the plasma is suppressed by EDTA.

Molecular Size and the Adhesive Activity of vWF

Laboratory and clinical observations suggest that large multimers are hemostatically more effective than small multimers. However, the basis for the dependence of hemostatic function of vWF on its multimer size is unclear. Furthermore, why is vWF secreted as a large polymer, only to be cleaved to smaller forms in the circulation? When evolution develops this circuitous scheme of vWF biosynthesis, it must be serving a biological function.

The responsiveness of the conformation of vWF to shear stress raises speculation that large multimers are hemostatically more effective because they are more responsive to shear stress. Presumably, large vWF multimers are more capable of supporting platelet aggregation because the con-
Formation of thrombi that are enriched in vWF and platelets, as are detected in patients with TTP,17 is predicted that a lack of ADAMTS13 should cause the formation of thrombi that are enriched in vWF and platelets, as are detected in patients with TTP. 17

**Deficiency of ADAMTS13 Causes TTP**

TTP, first described by Moschcowitz,18 typically presents in previously healthy individuals with thrombocytopenia and microangiopathic hemolysis, accompanied in advanced cases by fever and organ dysfunctions. 19 Neurological complications are the most common cause of death. The clinical manifestations are attributable to tissue injury resulting from thrombi in the capillaries and arterioles. Without treatment, the disease is fatal in >90% of cases. When treated with plasma infusion or plasma exchange, 60% to 90% of patients survive the acute episodes of TTP. 20, 21 Although TTP is relatively uncommon (~4 to 20 cases per 106 per year), the mysterious, abrupt development of platelet-rich thrombi in the arterioles and capillaries and the dramatic response to plasma infusion or exchange are intriguing. Relapse is common after the patients achieve remission. 22, 23

Previously, 3 lines of evidence suggested that vWF was involved in the pathogenesis of TTP: first, an increase or decrease in the size of vWF multimers was observed in patients with TTP, depending on the stage of the disease; second, the thrombi in TTP are enriched in vWF and platelets; and third, vWF is detected on the surface of platelets in patients with TTP. 25

**Autoimmune Inhibitors of ADAMTS13**

A severe deficiency of ADAMTS13 activity is detected in patients with TTP 26–28 but not in randomly selected hospitalized patients or in patients who have thrombocytopenia, hemolysis, or thrombosis due to other causes. 28 IgG isolated from patients with TTP suppresses the activity of ADAMTS13 in normal plasma. Serial studies demonstrate that decreased inhibitor titers and increased ADAMTS13 levels accompanied remission of TTP. These observations suggest that TTP is an autoimmune disease in which inhibitory antibodies suppress ADAMTS13 activity, leading to vWF-platelet binding and microvascular thrombosis.

Studies in large series of patients continue to support the association between ADAMTS13 deficiency and TTP. In 1 series, a severe deficiency of ADAMTS13 activity was
Medications such as ticlopidine occasionally induce autoimmunity. The clonal nature of the ADAMTS13 inhibitors in some individuals after infections with Epstein-Barr virus, inhibitors of ADAMTS13 are self-limited in genetically susceptible subjects. By analogy to the development of autoantibodies of the red blood cells induced by α-methyldopa.

The experience in patients with inhibitors of factor VIII suggests that protein replacement therapy is effective only in patients who have low inhibitor titers (<5 to 10 Bethesda units per milliliter). A retrospective analysis of the TTP patients who participated in the randomized trial conducted by the Canadian Apheresis Group revealed that the inhibitor titers of ADAMTS13 were indeed very low: whereas most (76%) patients in that study tested positive for inhibitors, only 2 of the 41 cases investigated had a titer >5 U/mL, and none had a titer >10 U/mL. The low titers of inhibitors explain why, in most cases, plasma therapy is effective in raising the activity level of ADAMTS13. 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 (<0.1 U/mL) and therefore do not have sufficient time to build up high titers. Alternatively but not exclusively, the inhibitors may be directed against other antigens yet are cross-reactive to ADAMTS13. Occasionally, the titer of inhibitors increases during the course of therapy, leading to treatment failure and a fatal outcome.

Genetic Deficiency of ADAMTS13
Schulman-Upshaw syndrome is a hereditary form of TTP with thrombocytopenia and microangiopathic hemolysis occurring soon after birth. These patients respond to plasma infusion, which is commonly repeated every 2 to 3 weeks to prevent the development of severe thrombocytopenia and complications. Deficiency of vWF cleaving protease was first described in 4 patients with chronic relapsing TTP. Ten cases with features of the Schulman-Upshaw syndrome have been studied genetically. Analysis of these patients from 7 pedigrees and their family members reveals that the protease level was <0.1 U/mL in the patients and partially deficient (0.49 to 0.68 U/mL) in the parents. The other genetically linked members in the family were equally divided between normal (0.79 to 1.27 U/mL) and partially deficient groups. The family members not genetically linked to the patients had normal protease levels, excluding environmental factors as the cause of ADAMTS13 deficiency. The severe deficiency of ADAMTS13 in the patients was corroborated by the presence of ultralarge vWF multimers in plasma. After infusion of 15 mL/kg of fresh frozen plasma, the protease level typically increased to ~0.2 U/mL and then declined to one half in ~2 days. The platelet count increased soon after plasma infusion, reached a maximum around day 7, and then declined to pretreatment levels by day 14. Some patients developed thrombocytopenia, microangiopathic hemolysis, and other complications only during periods of fever, infection, surgery, or pregnancy. Occasionally, thrombotic complications developed without apparent precipitating events.

Structure and Mutations of ADAMTS13
Linkage analysis of kindreds with hereditary TTP and amino acid sequencing of the purified protein have identified the protease as a member of the ADAMTS zinc metalloprotease family on chromosome 9. The deduced
The protein sequence of ADAMTS13 contains 1427 amino acid residues (Figure 5). The mature ADAMTS13 has a calculated polypeptide mass of 145 kDa, compared with the apparent mass of 190 kDa for the protease purified from human plasma. The difference is probably due to glycosylation: ADAMTS13 has 10 potential N-glycosylation sites, and the thrombospondin type I (TSP1) repeat has additional sites for other oligosaccharides.

Members of the ADAMTS family share several distinct protein modules, including a represin-like metalloprotease (M12B) domain with a zinc binding motif (HEXGHxxGxxHD), a disintegrin-like domain, and 1 or more TSP1 motifs. Individual members of the ADAMTS family differ in the number of C-terminal TSP1 motifs, and some have unique C-terminal domains, such as the CUB domain in ADAMTS13, which may be involved in substrate recognition. Among the 18 known human ADAMTS proteases, ADAMTS13 uniquely lacks a sequence motif in the propeptide similar to the “cysteine switch” of the matrixins (matrix metalloproteases) and adamalysins (ADAM). A sequence of RGDS that could bind to integrins on platelets or other cell types is present in the region following (ADAM). A sequence of RGDS that could bind to integrins on platelets or other cell types; and a spacer domain with no specific structural features. Another TSP1 module contains 7 additional TSP1-like repeats that have significant loss of conserved amino acid residues. Two CUB domains near the carboxyl terminus could be critical for substrate recognition. The approximate locations of the missense (A), frame-shift (B), and nonsense (X) mutations identified in some of the genetic studies are indicated.

**ADAMTS13 Activity in Other Types of Thrombotic Microangiopathies**

Thrombocytopenia and microangiopathic hemolysis occur in patients after infection with shiga toxin–producing microorganisms and with factor H mutations and occasionally in patients with metastatic cancers, bone marrow transplants, HIV infection, autoimmune collagen-vascular diseases, medications, disseminated intravascular coagulopathy, or pregnancy (Table 2).

Infection-associated HUS typically develops a few days after the onset of painful and usually bloody diarrhea caused by shiga toxin–producing *Escherichia coli*, most commonly of the O157:H7 serotype. The majority of children with the disease recover without the support of plasma infusion or exchange. The prognosis is much worse in elderly patients. Fibrin-rich thrombi are found in the renal glomeruli and occasionally in the small arteries of the kidneys or other organs. Abnormal shear stress is believed to be responsible for fragmentation of the red blood cells. Neurological abnormalities occur in 10% to 30% of the cases. Although *E coli* O157:H7–associated HUS differs from TTP in etiology and in the frequent development of severe renal failure, the overlapping clinical manifestations raise the speculation that TTP and HUS might have the same mechanism of thrombosis.

Further confounding the disease classification, TTP or HUS is also broadly applied to disorders that have the common features of microangiopathic hemolysis and thrombocytopenia. In some reports, the distinction between HUS...
and TTP is based on the presence or absence of renal dysfunction or neurological abnormalities. In this scheme, TTP and HUS are viewed as a single disease entity with different clinical manifestations. On the basis of this approach, a patient may have TTP on 1 occasion and HUS on another, depending on the severity of renal impairment and the absence or presence of neurological complications.47,48 This classification may give 2 diagnoses (TTP and HUS) to a patient who really has 1 disease; conversely, patients with different diseases may be grouped under 1 diagnosis. This approach also overlooks the possibility that TTP and HUS belong to the syndrome of thrombocytopenia and microangiopathic hemolysis that consists of a heterogeneous group of disorders (Figure 6).

Analysis of ADAMTS13 activity provides direct evidence that TTP is distinct in pathogenesis from other types of microangiopathic disorders. In a series of 16 cases of HUS after E coli O157:H7 infection, none of the patients had a severe deficiency of ADAMTS13 activity.13 In that series, none of the patients received plasma infusion or exchange, yet all recovered, underscoring the difference between TTP and HUS in pathogenesis and requirement of plasma therapy. Consistent with the experience described in previous series of E coli O157:H7–associated HUS, the severity of renal impairment was variable, and only 10 cases in this series required dialysis.

Serial samples obtained from these patients revealed that the onset of HUS was accompanied by a decrease in large vWF multimers and an increase in its proteolytic fragments.13 Presumably, thrombosis in the microvasculature increases the shear on vWF and enhances its cleavage by ADAMTS13. Histochemical studies of the thrombi in renal glomeruli detected the presence of fibrin but not vWF. These results suggest that in HUS, vWF is an innocent bystander rather than an active participant in the thrombotic process. Before the onset of HUS, patients with E coli O157:H7 infection typically exhibit an increase in the levels of F1+2, the prothrombin activation peptide, and D-dimer, a product of fibrin digestion by plasmin.49 This observation further strengthens the view that fibrin deposition, not vWF-platelet binding, leads to the development of HUS.

In a series of 29 cases of diarrhea-associated HUS in children, ADAMTS13 activity was present in all but 1 case.50 Overall, current evidence indicates that TTP and E coli O157:H7–associated HUS have distinct pathogeneses and require different therapies. However, a distinction between them cannot be made based on the severity of renal impairment or the presence of neurological abnormalities. Other studies reported low ADAMTS13 levels in patients with HUS and normal ADAMTS13 levels in patients with TTP.48,51 In these studies, the diagnosis of HUS was either provided by the referring physicians or based on the absence of neurological complications.

Among patients with plausible causes of thrombotic microangiopathy, the pathogenesis of thrombocytopenia and microangiopathic hemolysis is heterogeneous. Inhibitors of ADAMTS13 are detected in all of the ticlopidine-associated cases investigated50 and in 1 case of microangiopathic hemolysis soon after renal allograft transplantation.52 On the other hand, a severe deficiency (<0.1 U/mL) of ADAMTS13 is not detected in patients with metastatic cancers or bone marrow transplants.53–55 Thus, the potential role of ADAMTS13 deficiency in patients who have plausible causes of thrombotic microangiopathy needs to be investigated individually.

**Clinical Implications**

**Diagnosis**

The data reviewed here suggest that TTP may be defined as a thrombotic disorder resulting from a severe deficiency of...
ADAMTS13 activity (Table 3). This definition identifies patients who have a common disease mechanism and may potentially benefit from replacement of ADAMTS13 when this protein becomes available. Furthermore, this definition enables the diagnosis of TTP in atypical cases that present with thrombosis without the concurrent microangiopathic hemolysis and/or thrombocytopenia. Previously, it was impossible to make the diagnosis of TTP in patients without thrombocytopenia and microangiopathic hemolysis. A correct diagnosis of TTP by ADAMTS13 assay led to the successful management of recurrent episodes of transient cerebral ischemia in a patient who did not have hematological changes commonly associated with TTP.66

In a recent report, 3 siblings had a history of jaundice and thrombocytopenia during the neonatal period.67 After the propositus was found to have a deficiency of ADAMTS13, investigation of the family members revealed that her 2 older siblings also had the same disease that had been unrecognized previously. The experience of the kindred suggests that a genetic deficiency of ADAMTS13, particularly in the milder ones, is more common than previously appreciated.

Assay of ADAMTS13 activity is particularly useful in patients with multiple medical problems in whom the diagnosis of TTP is otherwise uncertain. Conversely, in a patient suspected of having TTP, a normal ADAMTS13 level should prompt search for other causes, such as occult metastatic cancers or autoimmune collagen vascular diseases that require different therapies. Potentially, a mutation in vWF that diminishes its susceptibility to ADAMTS13 cleavage could also result in the presence of ultralarge vWF multimers and the development of platelet thrombosis as encountered in TTP. However, just as patients with decreased large vWF multimers due to mutations in platelet glycoprotein Ib are not considered to have von Willebrand disease, thrombosis due to mutations of vWF or other unknown mechanisms should be considered separate disorders.

Implementation of this definition requires a reliable assay. To avoid potential errors that may result from laboratory measurements, a low ADAMTS13 value should be corroborated by evidence of diminished vWF cleavage and the presence of a cause of its deficiency, such as inhibitors of ADAMTS13 or genetic mutations.

Therapies

Plasma therapy is the only treatment with proven efficacy for patients with acute TTP. For patients with TTP due to mutations of the ADAMTS13 gene, infusion of small amount of fresh frozen plasma every 2 to 3 weeks is sufficient for preventing severe thrombocytopenia or other complications. Because of the presence of inhibitors of ADAMTS13, patients with the acquired form of TTP are best treated with plasma exchange. Plasma therapy is associated with the risks of transfusion reactions and transmission of infectious agents. Frequently it requires placement of central lines that are occasionally complicated with sepsis, thrombosis, or bleeding. ADAMTS13 protein is a potentially attractive alternative to fresh frozen plasma as the treatment for TTP.

Patients with refractory or relapsing TTP currently are treated with long-term plasma exchange, which is prohibitively expensive and debilitating to the patients. In some cases, remission is observed after the empirical use of corticosteroids, splenectomy, vincristine, cyclophosphamide, azathioprine, staphylococcal protein A columns, or high-dose immunoglobulins. However, in other patients, the disease persists despite these treatments. In preliminary series, rituximab, a chimeric monoclonal antibody of CD20 that depletes B cells in the circulation and lymph nodes, has shown promising results in patients with refractory TTP who failed the other conventional therapies.56,58

Assay of ADAMTS13 Activity

After the initial reports of an association between ADAMTS13 deficiency and TTP, several studies reported very low vWF cleaving activity in normal individuals, in patients without thrombotic microangiopathies, and in patients with HUS, raising questions on the validity of this association.59–61 In contrast, our studies found that the range of ADAMTS13 activity is narrow (0.79 to 1.27 U/mL) in normal subjects, and the level of the protease is either normal or slightly decreased in neonates and in patients with metastatic cancers, liver diseases, or heparin-induced thrombocytopenia.28,62,63

As discussed earlier, vWF is resistant to ADAMTS13 unless it is unfolded by shear stress. For technical reasons, the assays of ADAMTS13 activity use either urea (1.5 mol/L)26 or guanidine hydrochloride (0.15 mol/L)28 to promote the susceptibility of vWF to cleavage by ADAMTS13. One version of the assays also requires the addition of barium chloride.26 The potential contribution to assay variability by the experimental conditions has not been adequately explored. Furthermore, the methods for detecting vWF cleavage by ADAMTS13 are different. In our assay, vWF cleavage by ADAMTS13 is detected by measuring the fragments produced by ADAMTS13. In other assays, detection of vWF cleavage by ADAMTS13 is based indirectly on a decrease in vWF multimer size, a decrease in the collagen-binding activity of vWF, or a decrease in the vWF species that bind 2 monoclonal antibodies directed against vWF epitopes on the opposite sides of the cleavage.

Certain plasma factors may cause more interference of the assay under different experimental conditions. Also, in some plasma samples, the protease activity may deteriorate during storage. Although ADAMTS13 is stable at −70°C for many years in normal plasma,28 its stability in plasma samples of patients with pathological conditions has not been systemically investigated. Until these issues are resolved, a low assay value of ADAMTS13 should be interpreted with caution, particularly when it is not accompanied by evidence of diminished vWF cleavage and the presence of inhibitors or genetic mutations.

One report described the presence of normal vWF cleaving activity in a patient with thrombocytopenia, microangiopathic hemolysis, and ultralarge vWF multimers since early infancy and in her parents.64 Reanalysis of this family by our assay revealed that the patients had <0.1 U/mL ADAMTS13 activity, and both parents had partially decreased levels. The deficiency in this family was confirmed by DNA sequence analysis, which detected a homozygous dinucleotide deletion...
in the patient. The experience in this case demonstrates that hereditary TTP remains associated with a single locus and highlights the importance of prudence in interpretation of data when an ADAMTS13 activity assay produces unexpected data.

Summary and Future Directions

Current evidence suggests that high levels of shear stress promote the interaction between vWF and platelets. On the other hand, shear stress also increases the susceptibility of vWF to cleavage by ADAMTS13. By cleaving vWF, ADAMTS13 plays a pivotal role in regulating the interaction between vWF and platelets in the circulation.

Assay of ADAMTS13 activity is extremely useful in patients suspected of having TTP. Unfortunately, technical difficulties presently restrict the use of ADAMTS13 assays to research laboratories. Future challenges include the development of easy, reliable assays of ADAMTS13 as a potential alternative to fresh frozen plasma, and the use of immunotherapies to suppress inhibitors of ADAMTS13.

Plasma exchange, used in many patients with or without TTP, is associated with potentially serious complications. Studies are needed to delineate the role of plasma therapy in the management of patients without ADAMTS13 deficiency.

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