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Current Topics

Processing of von Willebrand Factor by ADAMTS-13†

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History

ADAMTS-13 is synonymous with von Willebrand factor cleaving protease, which was named for an activity in human plasma that specifically cleaves polymeric von Willebrand factor (VWF).[1] It was also called von Willebrand factor processing activity and the Upshaw factor. Recognition of the importance of ADAMTS-13 came from convergent investigations on the pathogenesis of a disease called thrombotic thrombocytopenic purpura (TTP), and the processing of mutant VWF molecules.

TTP is a rare thrombotic microangiopathic disease first reported by Moschcowitz in 1924 (*1*). It is characterized by widespread deposition of thrombi in the microvasculature, obstructing blood flow to organs, causing multiorgan failure and death (reviewed in ref *2*). Treatment by plasma exchange has reduced the mortality rate to less than 20% (*3*), and suggested that a component(s) in the plasma of TTP patients causes the disease. TTP is usually triggered by a precipitating event such as pregnancy, use of certain drugs, infection, or bone marrow transplantation, although the mechanism by which these seemingly unrelated events are associated with the disease is unknown. About half of the patients have recurrent episodes of the disease at unpredictable intervals, while a small fraction (2−3%) have an inherited chronic relapsing form of the disease called familial TTP that recurs predictably at intervals of 20−30 days.

Involvement of Unusually Large von Willebrand Factor

In 1982, Moake and co-workers observed that "unusually large" VWF multimers (UL-VWF) were present in patients with chronic relapsing TTP, who were in remission. This was the first indication that TTP is related to VWF, an adhesive plasma glycoprotein that plays an important role in primary hemostasis. VWF is synthesized in endothelial cells and megakaryocytes as prepro-VWF, and the presequence is proteolytically removed in the rough endoplasmic reticulum. Pro-VWF monomers assemble into homodimers and high multimers by formation of disulfide bonds. High polymers of VWF, with masses of >20 million Da, are further processed by a furin-like enzyme to remove the propeptide (*4*). This highly polymerized form of VWF, called proto-VWF, is stored in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells. Plasma VWF originates primarily from the Weibel-Palade bodies of endothelial cells. Upon secretion into plasma, proto-VWF is converted into a less polymerized form that includes dimers to 20-mers. Moake and co-workers hypothesized that patients with relapsing TTP have a deficiency of a depolymerizing activity, leading to the accumulation of proto-VWF or UL-VWF in circulation. The highly adhesive UL-VWF agglutinates platelets under high fluid shear in the absence of vascular damage to form pathological microvascular thrombi, which are deposited in the microcirculation throughout the body. The UL-VWF present in these patients during remission disappears after transfusion of fresh frozen plasma (*5*). Moake and co-workers hypothesized that the depolymerizing activity could be a protease or a disulfide bond reductase (*6*).

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[1] Abbreviations: ADAMTS, disintegrin-like and metalloprotease with thrombospondin type 1 motifs; VWF, von Willebrand factor; TTP, thrombotic thrombocytopenic purpura; UL-VWF, unusually large von Willebrand factor; TS1, thrombospondin type 1; CUB, C1r/C1s, urinary EGF and bone morphogenetic protein; HUS, hemolytic uremic syndrome; ADAMTSL, ADAMTS-like.

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Other lines of evidence suggest that limited proteolysis occurs to VWF polymers after their secretion into plasma. Infusion of 1-desamino-8-D-arginine vasopressin (DDAVP) in normal individuals causes an increased level of secretion from endothelial cells and a transient appearance of UL-VWF in plasma. These molecules rapidly disappear from circulation because of proteolysis (*7*). Studies on type IIA von Willebrand's disease showed that although the mutant VWF molecules are assembled normally into proto-VWF polymers in endothelial cells, they are rapidly and excessively degraded to dimers and trimers upon secretion into plasma (summarized in ref *8*). Excessive degradation and loss of polymeric structure cause bleeding (*9*, *10*). In the excessively degraded mutant VWF molecules, cleavage occurs at the Tyr^{1605}−Met^{1606} bond in the A2 domain [residues renumbered according to a recently proposed convention (*11*)]. In 1994, Tsai and co-workers identified a metalloprotease in plasma that cleaves normal polymeric VWF at the characteristic Tyr−Met bond in a shear-dependent manner (*12*). The unique and identical pattern of cleavage of normal VWF molecules under shear and the type IIA mutant VWF molecules suggests that the same metalloprotease is responsible for cleaving these molecules, regulating the polymer size of normal VWF molecules in one case, and excessively degrading the type IIA mutant molecules in the other.

von Willebrand Factor Cleaving Protease

Support for the UL-VWF theory was provided in 1996 by two studies (*13*, *14*), in which the plasma metalloprotease was partially purified. These studies showed that the metalloprotease is insensitive to inhibitors of matrix metalloprotease, serine, and sulfhydryl proteases, and has an apparent mass of ∼200−300 kDa. To cleave normal VWF efficiently, the substrate VWF molecules must first be partially denatured by exposure either to high fluid shear or to mild chemical denaturants such as 1 M urea or guanidine. A small amount of UL-VWF recovered from the conditioned medium of cultured endothelial cells was cleaved under high fluid shear or after exposure to guanidine by this protease to generate fragments identical to those from type IIA VWF mutants. This enzyme has a pH optimum of 8, and its activity is enhanced in low-ionic strength buffer in vitro. Ba^{2+}, Ca^{2+}, and Sr^{2+} ions are most effective in promoting the proteolytic activity; Mg^{2+} is slightly effective, and Zn^{2+} and Mn^{2+} are ineffective. The enzyme is inhibited by chelating agents such as EDTA, EGTA, and 1,10-phenanthroline, and its activity can be restored by Ca^{2+}. This protease was named VWF cleaving protease.

Further support for the involvement of this protease in TTP was provided in two subsequent studies, which showed that essentially all acute idiopathic TTP patients lack this proteolytic activity in their plasma and a majority of these patients have circulating autoimmune IgG inhibitors to the VWF cleaving protease (*15*, *16*). These findings support the hypothesis that the VWF cleaving protease plays an important role in the pathogenesis of TTP. After secretion into plasma, UL-VWF polymers are particularly sensitive to shear, which induces a conformational change in the molecule, exposing the cleavage site in the A2 domain to the VWF cleaving protease. Proteolysis is limited to UL-VWF, since VWF polymers of normal size are not sensitive to the level of shear stress in the arteriole circulation. Normal VWF polymers remain sensitive to higher shear stress when they are tethered to subendothelial components at the site of a vascular injury. Studies on type IIA mutant VWF molecules showed that mutations in the vicinity of the Tyr−Met cleavage site in the A2 domain of VWF probably induce a conformational change in this domain that exposes the cleavage site, resulting in excessive cleavage of the Tyr^{1605}−Met^{1606} bond by the VWF cleaving protease. A constitutional deficiency of the VWF cleaving protease in inherited TTP and an acquired deficiency of the VWF cleaving protease caused by failure in synthesis or the presence of autoimmune antibodies to the VWF cleaving protease would lead to accumulation of the UL-VWF polymers in circulation and TTP. Plasma exchange not only replenishes the missing VWF cleaving protease but also removes the autoimmune inhibitor antibodies if present. The concentration of VWF cleaving protease in plasma is estimated to be ∼1 μg/mL (*17*), and its half-life in vivo is ∼2−3 days (*18*).

Purification and Cloning

The VWF cleaving protease was purified to apparent homogeneity independently by two groups (*17*, *19*). Gerritsen and co-workers used human plasma as the starting material and immunoaffinity chromatography in the initial steps of the purification. The final preparation contains four polypeptides of 110, 130, 140, and 150 kDa, which share an identical N-terminal sequence of AAGGILHLGLLVAVG, suggesting that the four polypeptides have been partially degraded at the C-termini (*17*). Fujikawa and co-workers used human factor VIII/VWF concentrates as the starting material and conventional column chromatography to obtain a preparation that contains predominantly a single 190 kDa species. This protease contains an N-terminal sequence essentially identical to that determined by Gerritsen and co-workers (*19*). Fujikawa and co-workers also revealed that the gene encoding this protease is located on chromosome 9q34. Partial overlapping cDNA sequences in the EST database show that the VWF cleaving protease contains an adamalysin-like metalloprotease domain followed by a disintegrin-like domain and multiple thrombospondin 1 type I repeats. Thus, the VWF cleaving protease contains features of the ADAMTS family, which is named for a disintegrin-like and metalloprotease with a thrombospondin type 1 motif. Independently, Levy and co-workers used genome-wide linkage analysis to study four pedigrees with familial TTP using 382 polymorphic markers. They identified a close association of the gene responsible for familial TTP with the microsatellite marker D9S164 in the chromosome 9q34 region. Studies on genes in the vicinity of the D9S164 marker were focused on a candidate that encoded a novel metalloprotease, to which 12 mutations from the four familial TTP kindred were mapped. Full-length cDNA for the metalloprotease was cloned, and the protease, with characteristics of the ADAMTS subfamily, was designated ADAMTS-13 (*20*). (In accordance with the Human Gene Nomenclature Committee, the human gene symbol is ADAMTS13, while the protein is designated ADAMTS-13.) The N-terminal sequence of the metalloprotease domain is identical to those determined experimentally by Gerritsen et al. and Fujikawa et al. (*17*, *19*). The full-length cDNA sequence for ADAMTS-13 was also determined in other laboratories (*21*−*23*). These studies showed that the primary translation product consists of 1427

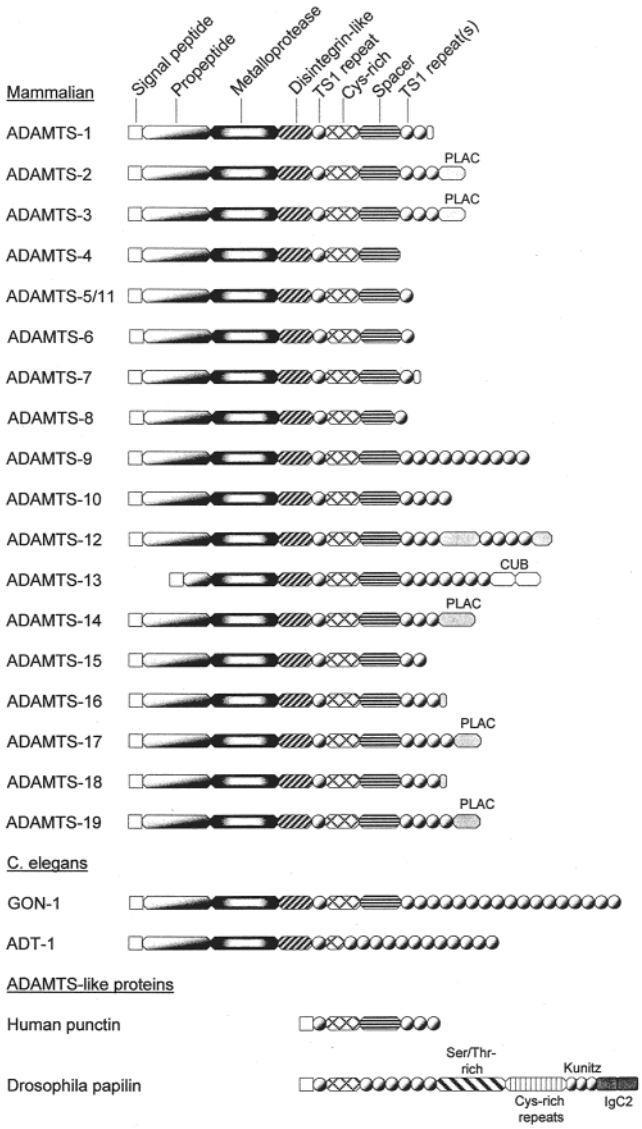

FIGURE 1: Organization of motifs in ADAMTS and related proteins: TS1, thrombospondin type 1; PLAC, protease lacunin; CUB, C1r/C1s, urinary EGF, bone morphogenetic protein; IgC2, immunoglobulin C2 loop domain.

amino acids organized in a series of motifs as shown in Figure 1. It contains an N-terminal signal peptide of 33 amino acids followed by a propeptide of 41 amino acids that ends with the sequence RQRR, a typical cleavage site for furin or a furin-like protease. The propeptide is followed by an adamalysin-like metalloprotease domain that begins with the experimentally determined N-terminal sequence of active ADAMTS-13. On the basis of sequence homology to adamalysin and the crystal structure of adamalysin II, two putative divalent cation binding sites in ADAMTS-13 have been proposed. A Zn^{2+} binding site, in which the Zn^{2+} ion is coordinated by three conserved His residues in the active site sequence $\underline{H}^{224}EIG\underline{H}^{228}SFGLE\underline{H}^{234}D$, is essential for activity. A second Ca^{2+} binding site, coordinated by Glu^{83}, Asp^{173}, Cys^{281}, Asp^{284}, and Met^{249}, may also contribute to enzymatic activity. The presence of both metal ion binding sites is consistent with inhibition of the enzymatic activity by chelators such as EDTA and 1,10-phenanthroline. The metalloprotease domain is followed by a disintegrin-like domain, a thrombospondin 1 repeat (TS1), a characteristic Cys-rich, and spacer sequence. The specific arrangement of these domains comprises the defining characteristic of the ADAMTS family of metalloproteases. In addition, individual members of the ADAMTS family (Figure 1) contain unique combinations of additional TS1 motifs and other motifs at the C-terminus. ADAMTS-13 contains seven additional TS1 repeats and two CUB domains. In addition to 10 potential N-glycosylation sites, the TS1 repeats in ADAMTS-13 contain the conserved sequence CSXSCG, which may be attachment sites for the O-linked disaccharide Glc-Fuc-(O-Ser) (*24*). The WXXW sequence in the first TS1 repeat may also be a site for C-mannosylation (*24*). Glycosylation presumably accounts for the difference between the calculated polypeptide mass (145 kDa) and the apparent mass of purified ADAMTS-13 from human plasma (190 kDa). ADAMTS-13 is also the only member of the ADAMTS family that contains CUB domains, which were first identified in the complement proteins C1r and C1s. Northern blot studies show that the mRNA for ADAMTS-13 is ~4.6 kb in size and is synthesized primarily in the liver (*20*−*22*), while a 2.4 kb transcript was also detected in skeletal muscle and placenta (*21*). The nature of the shorter transcripts is not known. Consistent with liver as the primary site of synthesis, partial ADAMTS-13 deficiency caused by congenital biliary atresia is corrected by liver transplantation (*25*). Recent studies also showed that the level of the ADAMTS-13 transcript was elevated in a colon carcinoma cell line (*23*). Sensitive reverse transcriptase PCR studies showed that trace amounts of the transcript for ADAMTS-13 were present essentially in all tissues (*21*). Alternative splicing of the ADAMTS-13 transcript gave rise to at least seven potential variants that were truncated at various positions after the metalloprotease domain (*20*, *21*). The four species isolated from plasma by immunoadsorption (*17*) might correspond to alternatively spliced forms of ADAMTS-13 in addition to being proteolytic fragments from larger species.

Activity and Specificity

The only known substrate for ADAMTS-13 is multimeric VWF, in which the Tyr^{1605}−Met^{1606} bond has been exposed by either high fluid shear or chemical denaturants. Crystal structures of the A1 and A3 domains of VWF have been determined (*26*, *27*), and the structure of the A2 domain has been predicted by molecular modeling (*28*). These studies suggested that the Tyr^{1605}−Met^{1606} bond was buried within the native A2 domain in a solvent inaccessible location, consistent with its insensitivity to ADAMTS-13 cleavage. Nine VWF type IIA mutations, which also occur in other buried locations, presumably disrupt folding of the A2 domain and cause exposure of the Tyr^{1605}−Met^{1606} bond to cleavage by ADAMTS-13.

The assay for ADAMTS-13 activity includes measuring the increase in the abundance of characteristic VWF fragments or the loss of VWF polymers after digestion. Tsai and co-workers measured the shear-dependent increase in the abundance of fragments of 200 and 350 kDa, which upon reduction, were shown to be homodimers of the N-terminal 140 kDa and C-terminal 176 kDa fragments of the VWF subunit (*12*). The nature and abundance of the degradation fragments were detected by antibodies in Western blotting. Furlan and co-workers measured the extent of the loss of

VWF polymers after normal VWF polymers were partially denatured with 1.5 M urea and digested with ADAMTS-13. VWF polymers were displayed on SDS−agarose gels and detected by Western blotting (*29*). Gerritsen and co-workers measured the residual binding capacity of digested VWF to immobilized collagen (*30*). Binding of VWF to collagen depends on its polymeric structure. Loss of VWF polymers after digestion with ADAMTS-13 causes a corresponding loss in the level of collagen binding. Residual VWF binding to collagen was detected by an enzyme-linked immunosorbant assay using anti-VWF antibodies. A modification of the collagen binding assay was devised by Aronson and co-workers, who used the test plasma as a source of both VWF and ADAMTS-13 (*31*). VWF in test plasma was first partially denatured by 1.5 M urea and then cleaved by ADAMTS-13 in the same sample. After digestion, the amount of residual polymeric VWF was measured by collagen binding and an enzyme-linked immunosorbant assay. Fujikawa and co-workers used VWF fragment III (Ser^{764}−Glu^{2128}) as the substrate for ADAMTS-13 (*19*). VWF fragment III was derived from VWF by cleavage with staphylococcal V-8 protease (*32*). Digestion of VWF fragment III by ADAMTS-13 gave rise to products of 140 and 65 kDa from the N- and C-termini of fragment III, which were identified by Western blotting. The unique appearance of the 65 kDa fragment avoided the high background presence of the 176 and 140 kDa fragments, since a factor VIII/VWF concentrate was used as the source of ADAMTS-13. Obert and co-workers used a two-site immunoradiometric assay to quantify residual uncleaved VWF (*33*). Monoclonal antibodies specific for an epitope C-terminal to the cleavage site were used to capture VWF molecules, and loss of N-terminal fragments resulting from digestion with ADAMTS-13 was detected by a decreased level of binding of ^{125}I-labeled monoclonal antibodies specific for the N-terminus of the VWF subunit.

Hemolytic Uremic Syndrome

Hemolytic uremic syndrome (HUS) often exhibits symptoms similar to those of TTP, and both diseases show manifestations of microvascular platelet aggregation and red cell mechanical breakage. Although the microthrombi associated with HUS primarily occur in the renal circulation, extrarenal complications may obscure unequivocal differentiation of the two syndromes. Recent studies show that ADAMTS-13 activity is normal in familial or acquired HUS (*15*), suggesting that quantitative measurement of the ADAMTS-13 activity may distinguish between TTP and HUS.

Disulfide Reductase

Although ADAMTS-13-mediated proteolysis can account for the depolymerization of UL-VWF, the role of disulfide bond reduction is not clear. Phillips and co-workers identified a disulfide bond reductase in human plasma that depolymerizes UL-VWF to a less polymerized form (*34*). However, plasma from familial TTP patients contains normal levels of this enzyme, indicating that this activity alone is insufficient for depolymerizing UL-VWF in vivo (*35*). Another reductase activity was found in the conditioned medium of several types of cultured endothelial cells. Reduction in VWF polymer size was dependent on Ca^{2+} and was accompanied by the appearance of free thiol groups in VWF (*36*). This reductase was purified from the conditioned medium of cultured human dermal microvascular endothelial cells and shown to be thrombospondin 1 (*37*). A transient intermediate of the reaction, consisting of disulfide-linked VWF and thrombospondin 1, was trapped by the addition of *N*-ethylmaleimide. The extent of VWF reduction, shown by an increase in the number of free sulfhydryl groups in VWF, was proportional to the amount of thrombospondin 1 added. The plasma thrombospondin 1 levels in three TTP patients were significantly reduced. Intraperitoneal injection of human thrombospondin 1 in mice decreased the polymer size of mouse VWF in circulation. The physiological significance of this activity and its relation to TTP requires further investigation.

The ADAMTS Family and Related Proteins

In *Caenorhabditis elegans*, cells at the distal tips of the two growing arms of the U-shaped hermaphrodite gonad determine the shape and development of the organ. Migration of these cells is regulated by the *gon-1* gene, which encodes a secreted metalloprotease of the ADAMTS family (*38*). In addition to the signal, prometalloprotease, disintegrin-like, TS1, Cys-rich, and spacer sequences, GON-1 also contains 17 additional TS1 repeats at its C-terminus. Failure of the distal tip cells to migrate in *gon-1* mutants led to agenesis of the organ. GON-1, together with another metalloprotease, MIG-17 (*39*), may cleave components in the extracellular matrix to expose signals that guide migration of the distal tip cells and organ development. The *adt-1* gene in *C. elegans* also encodes an ADAMTS metalloprotease that is important in the morphogenesis of the male-specific organs (*40*). ADT-1 contains 12 additional TS1 repeats at its C-terminus in addition to the signature motifs of the ADAMTS family, although it contains a truncated Cys-rich sequence. ADT-1 lacks a spacer sequence.

Since the discovery of the first mammalian ADAMTS in 1997, a total of 18 members have been identified. These were designated ADAMTS-1−19, while ADAMTS-5 and ADAMTS-11 were literature aliases. Recent members were identified by human genome searches using the defining characteristics of this family (*23*), while the metalloproteases have not been isolated or characterized. In addition to ADAMTS-13, the functions of only five other mammalian ADAMTS metalloproteases were known. The level of expression of ADAMTS-1 was elevated by inflammation (*41*), and transgenic knockout of this gene in mouse led to infertility, abnormal growth, and impairment in ureteral and adrenal developments (*42*). ADAMTS-1 binds to the extracellular matrix, and this binding depends on the spacer sequence and the three TS1 repeats (*43*). ADAMTS-1 inhibits angiogenesis and is further processed in the extracellular matrix by matrix metalloproteinases 2, 8, and 15 to release two C-terminal TS1 repeats (*44*). Removal of the C-terminal TS1 repeats may modulate the antiangiogenic properties of ADAMTS-1. ADAMTS-2, also called procollagen I *N*-proteinase, removes the N-terminal propeptides from both type I and type II procollagen subunits and is necessary for collagen fibril assembly (*45*). Mutations in ADAMTS-2 cause Ehlers-Danlos syndrome type VIIC in humans and dermatosparaxis and fragile skin in animal species (*46*). ADAMTS-3 is also a processing enzyme for procollagen. It

is highly expressed in cartilage and removes the N-terminal propeptide from type II procollagen (*47*). ADAMTS-4 and ADAMTS-5 (ADAMTS-11) are aggrecanases, which degrade aggrecan in cartilage in degenerative arthritic diseases (*48*). Cleavage of aggrecan releases peptides that contain glycosaminoglycan, resulting in a loss of compressive properties in the cartilage. ADAMTS-4 also cleaves other proteoglycans such as brevican (*49*) and versican (*50*). The substrates and functions of the remaining ADAMTS proteases are unknown.

Drosophila papilin is an ADAMTS-like protein that lacks the prometalloprotease and disintegrin-like domain, but contains a signal peptide directly linked to the first TS1 repeat, followed by a Cys-rich and spacer sequence, six additional TS1 repeats, a Ser/Thr-rich region, 12 short Cys-rich repeats, three Kunitz-like domains, two Ig-C2 loop domains, and a C-terminal peptide (*51*). Papilins in *C. elegans* and mammalian species have different numbers of TS1 repeats and Kunitz-like domains. Papilin is localized to the extracellular matrix and is essential for organogenesis.

Punctin is another ADAMTS-like protein that lacks the prometalloprotease and disintegrin-like domains (*52*). Punctin, also called ADAMTSL-1, appears to be localized to the extracellular matrix, and its function is unknown.

Many metalloproteases from snake venom that possess hemorrhagic activities have been isolated. A unique metalloprotease named kaouthiagin, isolated from the venom of the cobra *Naja kaouthia*, cleaves the $Pro^{1471}-Asp^{1472}$ bond in the A1 domain of VWF. Unlike ADAMTS-13, kaouthiagin does not require fluid shear or partial denaturation to expose its cleavage site in VWF. Cleavage of VWF by kaouthiagin causes a loss of multimeric structure, and a corresponding loss of platelet and collagen binding capacity. Kaouthiagin is a metalloprotease of 401 amino acids and is composed of a zinc metalloprotease domain, a disintegrin-like domain, and a Cys-rich domain (*53*). Both the disintegrin-like and the Cys-rich domains inhibit collagen-induced platelet aggregation and possess disintegrin-like function.

Perspective

Recognition of the role of ADAMTS-13 in TTP represents a first step in understanding the pathogenesis of this disease. It is possible that recombinant or enriched preparations of ADAMTS-13 would replace plasma exchange in the treatment of TTP in the future. Development of rapid and sensitive assays for ADAMTS-13 would enable close monitoring of how its activity changes in the course of the disease and in response to treatment. Early recognition of prethrombotic conditions with these tests would significantly minimize organ damage and shorten the recovery period.

Currently, little is known about the regulation of ADAMTS-13 synthesis, especially events that would transiently downregulate its synthesis to cause TTP. The mechanism by which seemingly unrelated events such as pregnancy, use of antineoplastic drugs, and infection can trigger TTP is not known. It is also not well understood why transient autoimmune antibodies to ADAMTS-13 develop in some TTP patients, and how immunosuppression may affect the outcome, duration, and recurrence of the disease. It is hoped that these and other important questions about ADAMTS-13 and TTP will be the focus of future investigations and that a better understanding of the underlying mechanisms will suggest alternative and improved therapy.

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