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THROMBOTIC THROMBOCYTOPENIC PURPURA: SURVIVAL BY "GIVING A DAM"

JOEL L. MOAKE, M.D.

HOUSTON, TEXAS

ABSTRACT

A teenager died suddenly in 1923 of systemic microvascular thrombosis. Dr. Eli Moschcowitz attributed the "hitherto undescribed disease" (now "thrombotic thrombocytopenic purpura," or "TTP") to "some powerful poison" with "both agglutinative and hemolytic properties." In 1982, TTP was found to be a defect in the "processing" of unusually large (UL) von Willebrand factor (VWF) multimers. By 1998, the cause of TTP was known to be either familial absence or acquired inhibition (by autoantibody) of plasma VWF-cleaving metalloprotease. This enzyme, the 13th member of *a d*isintegrin *a*nd *m*etalloprotease family with *t*hrombospondin domains (ADAMTS-13), circulates in normal plasma waiting to cleave the long strings of ULVWF multimers emerging from stimulated endothelial cells. Uncleaved ULVWF multimers in TTP induce platelet adhesion and aggregation in the rapidly flowing blood of microvessels. Episodes of TTP are treated by "giving A DAM" (TS-13, that is) contained in normal plasma, either by infusion alone or in combination with plasmapheresis.

A "NEW DISEASE"

In 1924–5, Dr. Eli Moschcowitz of New York City described a 16-year-old girl with the abrupt onset and rapid progression of petechiae, pallor, paralysis, coma and death (1,2). Terminal arterioles and capillaries were occluded in the doomed teenager by "hyaline" thrombi that were later determined to consist mostly of platelets. No perivascular inflammation or endothelial cell damage was detectable. Dr. Moschcowitz suspected a "powerful poison which had both agglutinative and hemolytic properties" as the cause of this disastrous "new disease" (2) —today known as "thrombotic thrombocytopenic purura (TTP)."

Mailing address: J. L. Moake, M. D. Medical Hematology Section The Methodist Hospital Mail Station 902, Main Bldg. 6565 Fannin Street Houston, Texas 77030 (713) 348-5357 e-mail: jmoake@rice.edu

A HALF-CENTURY OF MYSTERY AND MORTALITY

For more than fifty years after the report of the first patient, and first death, the mortality in TTP hovered near 100%. During this frustrating period, definition of the dreaded disorder became progressively more precise. TTP is a severe microvascular occlusive "thrombotic microangiopathy." It is characterized by systemic platelet aggregation, organ ischemia, profound thrombocytopenia (with increased marrow megakaryocytes), and fragmentation of erythrocytes (3). The red blood cell fragmentation occurs as blood flows through turbulent areas of the microcirculation that are partially occluded by platelet aggregates, producing "microangiopathic hemolytic anemia" and schistocytes ("cut" red cells) on peripheral blood smears. Serum levels of lactate dehydrogenase (LDH) are elevated many-fold as a consequence of the leakage of LDH from ischemic or necrotic tissue cells (4). The systemic platelet clumping is often associated with blood platelet counts that are less than 20,000/μl. Occlusive ischemia of the brain or the gastrointestinal tract is especially common.

Thirty years ago, a "pentad" of signs and symptoms was believed to be associated commonly with TTP: thrombocytopenia; microangiopathic hemolytic anemia; neurologic abnormalities; renal failure; and fever (5). In 2003, however, thrombocytopenia, schistocytosis, and an extremely elevated serum LDH level are enough to suggest the diagnosis (3).

It is now clear that there are specific types of TTP. Familial TTP is the rarest. It may appear initially in infancy or childhood, and then recur (in the current era of effective therapy) at regular intervals of about 3-weeks ("chronic relapsing TTP") (3,6–8). Acquired ("out-of-the-blue") TTP, also once rare, is now common in adults and older children—for reasons unknown. Following successful treatment of acquired TTP, recurrent episodes at irregular intervals occur in 11–36 % of patients (3,9–12). Occasional patients treated for arterial thrombosis with the platelet adenosine diphosphate receptor-inhibiting thienopyridine drugs, ticlopidine (Ticlid) or structurally similar clopidogrel (Plavix), develop TTP within a few weeks after the initiation or therapy (3,13–15). TTP occurs infrequently during the last trimester of pregnancy or in the immediate post-partum period (3,16–18).

THE OVERLOOKED CLAIM

A prescient discussion about the young "TTP" patient of Dr. Moschcowitz ensued after her tragic death. Dr. Max Lederer, a local contemporary of Dr. Moschcowitz, announced that he had previously seen four

similar patients, and claimed that each recovered following a blood transfusion (2,19). This tantalizing therapeutic hint was then buried for years beneath treatment failures, frustration and bluster. In 1992, Dr. Aaron Marcus wrote (19):

> "Articles or conferences on any aspect of TTP frequently give rise to a barrage of Letters to the Editor consisting of protests about not being quoted, anecdotal cases in almost everyone's practice, and, of course, new therapies. There may also be complaints that the respondent had originated the proposed therapeutic approach 10 years previously."

THERAPEUTIC RE-DISCOVERY

Occasional optimistic reports touting the value of exchange transfusion in TTP (20) culminated in a description of hematologic remission achieved in three patients by Pisciotta, et al. in 1977 (21). In that same year, other empiric observations suggested that plasma-based therapy for TTP might be the long-sought therapeutic "breakthrough." Byrnes and Khurana (22) found that relapses of TTP in a single patient were consistently prevented or reversed by the infusion of fresh-frozen plasma or its cryoprecipitate-poor fraction (cryosupernatant). Bukowski, et al. described complete recovery in two TTP patients following intensive plasmapheresis (23). By 1981 (24), hematologists were hopeful that the concurrent infusion of normal fresh-frozen plasma and plasmapheresis (i.e., plasma exchange) might be the most consistently effective therapy of all in adult patients with acquired "out-of-the-blue" TTP. Although the pathophysiology of TTP remained totally mysterious for one more year, the diagnosis no longer carried an automatic death sentence.

THE LABORATORY SURPRISE

In 1982, "unusually large" (UL) von Willebrand factor (VWF) multimers were found in plasma samples obtained from four patients with familial chronic relapsing TTP (8). It was proposed that the ULVWF multimers induced the pathological systemic platelet clumping and, in fact, were the elusive "agglutinative" substances mentioned 58 years earlier by Moschcowitz (Figure 1). The 1982 report concluded that patients with chronic relapsing TTP are likely to have a defect in the "processing" of ULVWF multimers, making them susceptible to periodic TTP relapses (8). Supporting evidence for ULVWF multimers as the platelet-clumping culprits in TTP appeared in steady dollops for the next 15 years (6,25–29). The contention that failure to degrade

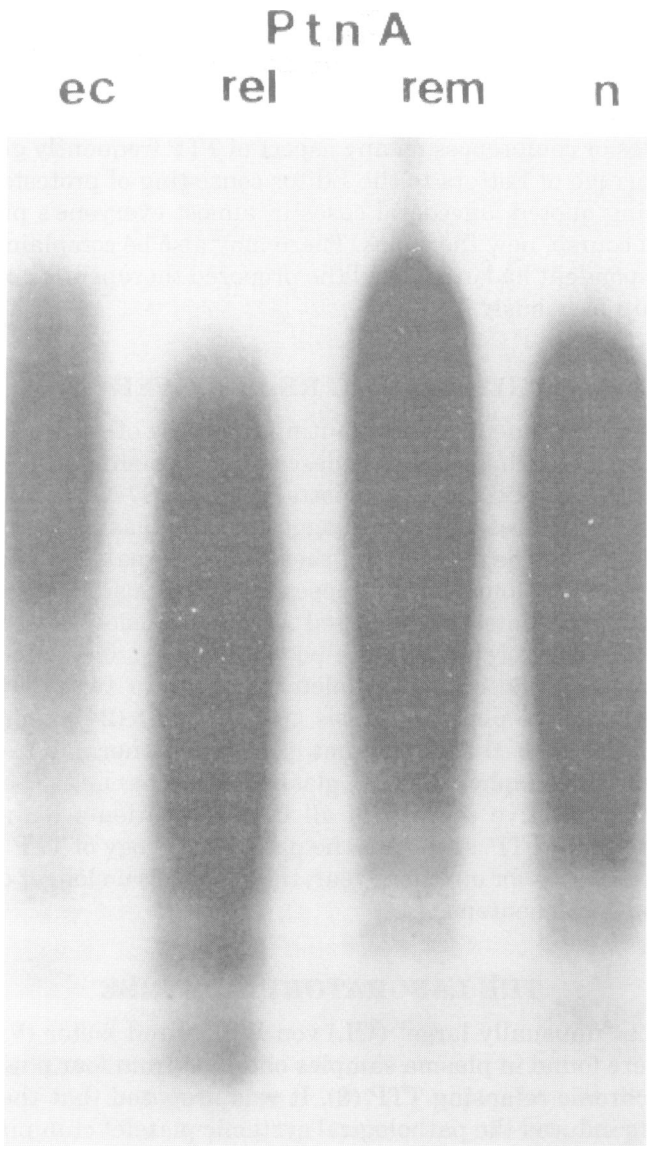


FIG. 1. Sodium dodecyl sulfate (SDS)-1% agarose gel electrophoretic analysis of von Willebrand factor (VWF) multimers in the plasma of patient A (Ptn A) with chronic relapsing TTP, and in the supernatant of cultured normal human umbilical vein endothelial cells. Normal pooled plasma = n; endothelial cell supernatant = ec; relapse = rel (platelets, 32,000/ul); remission = rem (platelets, 190,000/μl). After electrophoresis, the gel was fixed, the SDS removed, ^{125}I-anti-VWF overlaid, and the autoradiogram prepared. From reference (8).

ULVWF multimers causes the familial chronic relapsing, as well as the acquired types of TTP (8,25,28) (Figure 2) was almost submerged, however, beneath waves of vigorous dissent.

"VWF-PROCESSING ACTIVITY" DEFINED

Critical experiments confirming the above concept of TTP pathophysiology appeared in 1997–8. In 1997, Furlan, et al. (7) described four patients with chronic relapsing TTP who had a chronic deficiency of VWF-cleaving protease activity in plasma. Because no inhibitor of the enzyme was detected, the deficiency was ascribed to an abnormality in the production, survival or function of the protease. The following year, elegant papers by Furlan, et al. (30,31) and Tsai and Lian (32) demonstrated that VWF-cleaving metalloprotease activity was absent (or barely detectable) during acute episodes in the citrate-plasma of patients with acquired TTP. The activity returned to normal as the patients recovered. An IgG autoantibody against the VWF-cleaving metalloprotease enzyme probably accounted for the lack of protease activity in most of the acquired TTP patients reported in 1998 (32). The reasons for the transient immune dysregulation, as well as for the selective antigenic targeting of the VWF-cleaving metalloprotease, was not known then—or now. VWF "processing activity" is, therefore, a specific VWF-cleaving metalloprotease in normal plasma that cleaves ULVWF multimers and prevents their entrance into the circulation (or persistence). The VWF-cleaving metalloprotease was identified precisely in 2001 as the 13th member of a family of 18 ADAMTS-type enzymes identified to date, i.e., "ADAMTS-13". ADAMTS is the acronym for *a d*isintegrin *a*nd *m*etalloprotease with *t*hrombospondin-1-like domains (33–36).

THE SUCCESS OF EMPIRIC THERAPY EXPLAINED: "GIVING ADAM (I.E., TS-13)"

It was demonstrated in 1985 that the processing of ULVWF multimers could be restored in patients with familial chronic relapsing TTP by transfusing normal fresh-frozen plasma or cryosupernatant (25). In 1994, it was shown that this effect could also be obtained by infusing normal plasma that had been treated with solvent and detergent to inactivate viruses with lipid envelopes (HIV, hepatitis B and C) (6). Tsai and Lian (32) and Furlan, et al. (30) demonstrated in 1998 that the therapeutic component in these plasma products is the active VWF-cleaving metalloprotease enzyme (ADAMTS-13) missing or inhibited in the plasma of patients with TTP.

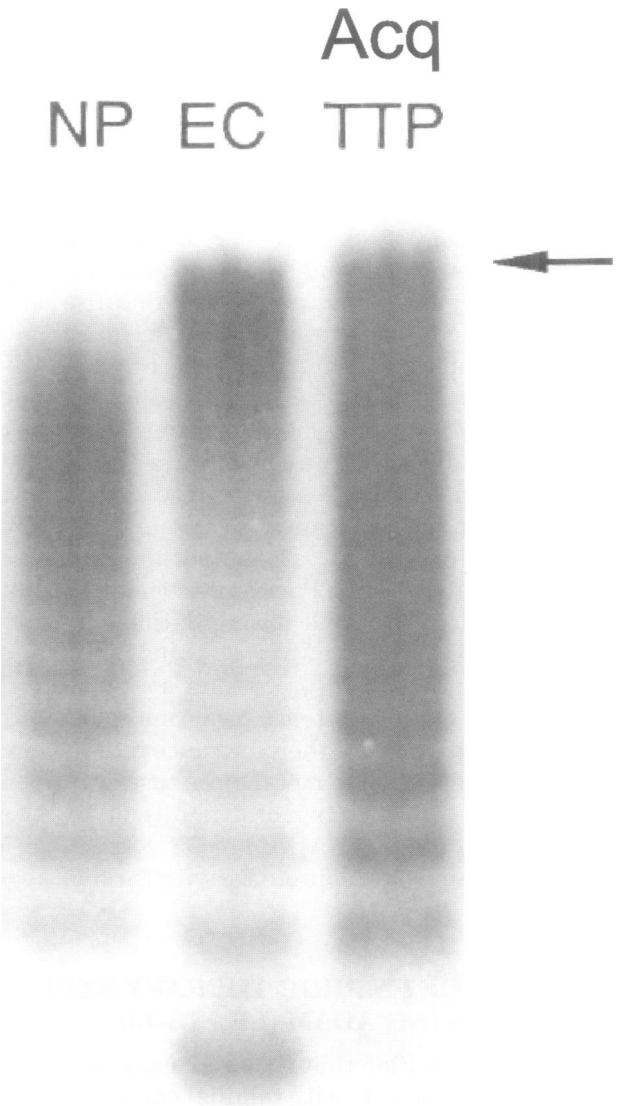


FIG. 2. Autoradiogram demonstrating unusually large (UL) von Willebrand factor (VWF) multimers in the EDTA-plasma of an adult patient early during a single episode of acquired (Acq) TTP. NP = normal pooled EDTA-plasma; EC = supernatant of cultured human umbilical vein endothelial cells containing ULVWF forms. Samples were mixed with SDS-urea-EDTA-Tris for electrophoresis in SDS-1% agarose. Display of VWF multimers was by incubation with ^{125}I-anti-VWF antibodies, and then exposure to x-ray film. Arrow at right indicates position of ULVWF multimers. From reference (74).

Adults and older children with acquired "out-of-the-blue" TTP episodes associated with ADAMTS-13 deficiency require daily plasma exchange. The plasmapheresis component of plasma exchange may remove circulating ULVWF multimers and attached platelets, as well as autoantibodies against ADAMTS-13. The fresh-frozen plasma or cryosupernatant infused contain uninhibited ADAMTS-13. Plasma exchange allows about 80–90% of patients with acquired TTP to survive an episode, usually without persistent overt organ damage (9,10).

TTP: MULTIMERS TO METALLOPROTEASE TO ADAMTS-13 DEFICIENCY

Monomers of VWF (280,000 Daltons) are linked by disulfide bonds into multimers with varying molecular masses that range into the millions of Daltons (37) (Figure 3). Plasma VWF multimers are smaller metabolic derivatives of the immense ULVWF multimers that are

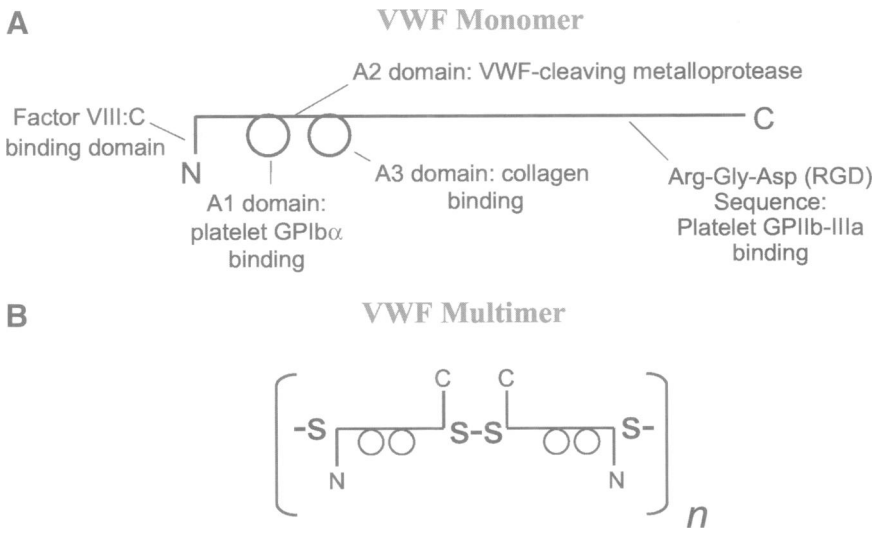


FIG. 3. Von Willebrand factor (VWF) monomers (280,000 Daltons) (A) are synthesized predominantly in vascular endothelial cells, and linked by disulfide bonds into dimers. The dimers are polymerized into disulfide-linked multimers of varying sizes (indicated by *n*) that range into the millions of Daltons (B), and stored in the Weibel-Palade bodies of endothelial cells. Each VWF monomeric subunit within the VWF multimer contains separate domains with binding sites for coagulation factor VIII, collagen, the glycoprotein (GP) Ibα component of platelet GPIb-IX-V receptors, and activated platelet GPIIb-IIIa complexes. VWF multimers, in a range of sizes that includes unusually large forms, are secreted by vascular endothelial cells backward into the collagen-rich subendothelium and forward into the plasma.

secreted in long string-like structures from the Weibel-Palade bodies of endothelial cells (8). ULVWF multimers, in contrast to plasma VWF multimeric forms, bind to the glycoprotein Ibα components of platelet glycoprotein Ib-IX-V receptors in the absence of artificial chemical or biological agonists (26,38). The initial attachment of ULVWF multimers to glycoprotein Ibα receptors, and subsequently to activated platelet glycoprotein IIb-IIIa complexes, induces platelet adhesion and aggregation under flowing conditions (26,27,38–40).

Plasma ADAMTS-13 is composed of an amino-terminal reprolysin-type metalloprotease domain followed by: a disintegrin domain; a thrombospondin-1-like domain; a cysteine-rich domain containing an arginine-glycine-aspartate (RGD) sequence; a spacer domain; seven additional thrombospondin-1-like domains; and duplicated CUB domains at the carboxyl-terminal end of the molecule. (CUB domains contain peptide sequences similar to: the *C*omplement subcomponents, C1r/C1s; the embryonic sea *U*rchin protein, egf; and *B*one morphogenic protein-1) (41) (Figure 4). ADAMTS-13 is a Zn^{2+} and Ca^{2+}-requiring 190,000 Dalton glycosylated protein that is encoded on chromosome 9q34, and produced predominantly in the liver (33–35,42–44).

The long string-like ULVWF multimers are cleaved by ADAMTS-13 as they are secreted from endothelial cells (45–48) (Figure 5). It is likely that ADAMTS-13 performs this function preferentially on or near the surface of endothelial cells, cleaving ULVWF multimers as they are released. At least one of the thrombospondin-1-like domains and one or both of the repeated CUB domains at the carboxyl-terminal end of each ADAMTS-13 enzyme molecule may be required to bind the enzyme to ULVWF multimers as they are secreted by endothelial cells (46,49). Specifically, ADAMTS-13 enzymes may attach to accessible A3 domains in the monomeric subunits of ULVWF multimers (46), and then cleave tyrosine 842–843 methionine peptide bonds in adjacent A2 domains (Figure 3). ADAMTS-13 may also interact with an (as yet uncharacterized) endothelial cell surface site in order to optimize its

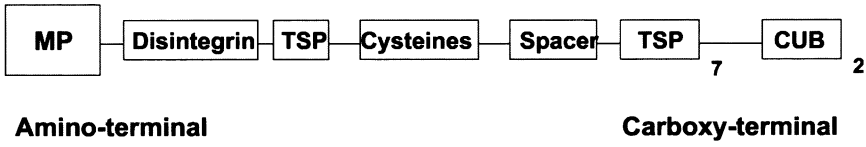


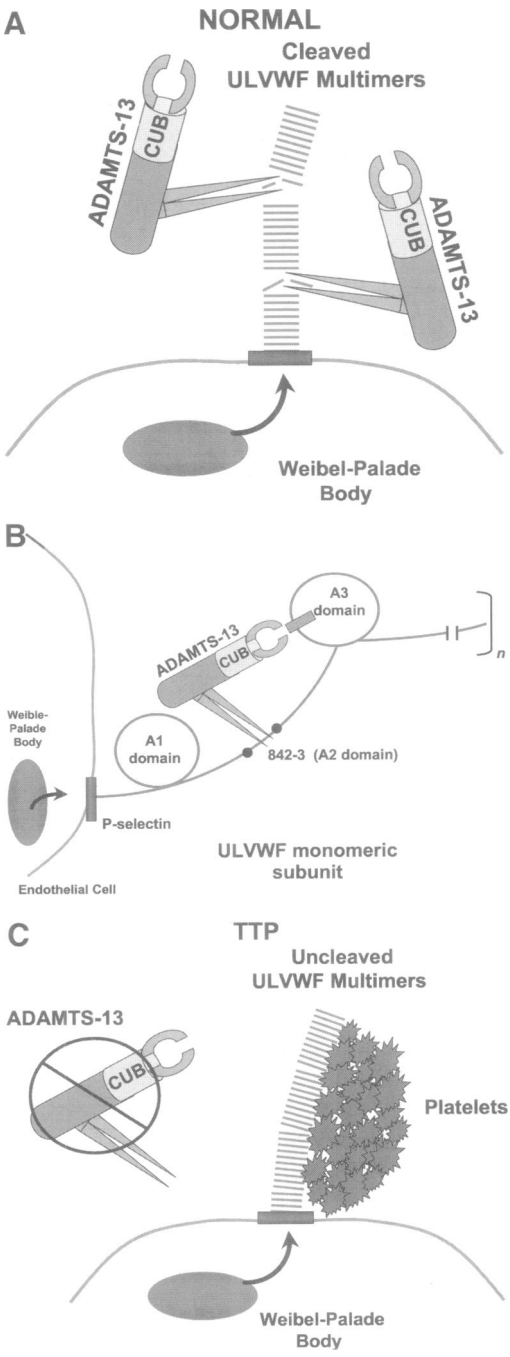
FIG. 4. Domain structure of the plasma VWF-cleaving metalloprotease, ADAMTS-13. MP, metalloprotease (proteolytic) domain; TSP, thrombospondin-1-like domain (a total of 8 are present); Cysteines, cysteine-rich domain; CUB, duplicated domain containing proteins similar to complement components, C1r/C1s, a sea urchin protein, and a bone morphogenic protein.

activity. Partial unfolding of the emerging long ULVWF multimeric strings by the fluid shear stresses in flowing blood may increase the efficiency of ADAMTS-13 attachment to ULVWF multimers, as well as subsequent ULVWF cleavage (45,50).

Patients with familial chronic relapsing TTP almost always have ULVWF multimers in their plasma, especially between episodes when the ULVWF forms are (for reasons unknown) less actively attaching to platelets (8,28) (Figure 1). ULVWF multimers are also detectable using a sensitive gel electrophoresis method in some patient plasma samples during acute episodes of acquired TTP, but not after recovery (28). The explanation for these findings was suggested by Furlan, et al. (7,30,31) and Tsai and Lian (32) to be a chronic absence of the VWF-cleaving protease from plasma in familial chronic relapsing TTP; and transient inhibition of the enzyme during acute episodes of acquired TTP.

Most patients with familial TTP have less than about 5% of normal ADAMTS-13 activity in their plasma, regardless of when the plasma is obtained (i.e., during or after acute episodes). Most patients with acquired types of TTP have less than about 5% of normal of ADAMTS-13 activity in their plasma only during acute TTP episodes (3,7,15,30–32,51,52). The severe deficiency of ADAMTS-13 activity in TTP patient plasma correlates with a failure to cleave ULVWF multimers as they emerge from the surface of endothelial cells (45) (Figure 3). As a consequence, ULVWF multimers remain anchored to the endothelial cells in long strings (45,46). The anchoring may be via P-selectin molecules, which have transmembrane domains and are secreted along with ULVWF multimers from the endothelial cell Weible-Palade bodies (53). Passing platelets adhere via their glycoprotein Ibα receptors to these long ULVWF multimers (45). Many additional platelets flowing in the bloodstream subsequently adhere and aggregate onto the ULVWF multimeric strings to form large, potentially occlusive, platelet thrombi. Platelets do not adhere to the smaller VWF forms that circulate after cleavage of ULVWF multimers (37).

ULVWF multimeric strings are capable of detaching from endothelial cells in the absence of ADAMTS-13 activity, the presence of fluid shear stress, and the increasing torque generated as platelets adhere and aggregate onto the ULVWF strings (45,46). The detached ULVWF-platelet strings may "embolize" to microvessels downstream and contribute to organ ischemia. The ULVWF multimeric component of the ULVWF-platelet strings that can sometimes be detected in TTP plasma samples by gel electrophoresis provided the initial clue to TTP pathophysiology.


A
NORMAL
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ULVWF Multimers
ADAMTS-13
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CUB
n
Weible-
Palade
Body
A1
domain
842-3 (A2 domain)
P-selectin
ULVWF monomeric
subunit
Endothelial Cell
C
TTP
Uncleaved
ULVWF Multimers
ADAMTS-13
CUB
Platelets
Weibel-Palade
Body

Plasma ADAMTS-13 activity is absent or severely reduced in most familial TTP patients (7,30,54) as a consequence of homozygous (or double heterozygous) mutations in each of the two ADAMTS-13 9q34 genes (34). Episodes of TTP usually commence in infancy or childhood. Occasionally, however, TTP episodes do not develop for years (as during a first pregnancy) in related individuals who lack plasma enzyme activity (16,52). One possible explanation for this latter clinical variant may be that the effectiveness of *in vivo* ADAMTS-13 cleavage of ULVWF multimers at the surface of endothelial cells exceeds the estimate of plasma enzyme activity in these particular patients using *in vitro* assays done under non-physiologic conditions. Alternatively, accentuated secretion of ULVWF multimers by endothelial cells under stimulation by estrogen or other agents may be required to provoke TTP episodes in some patients with severe deficiencies of plasma ADAMTS-13 activity.

Many patients with acquired TTP also have absent or severely reduced plasma ADAMTS-13 activity during an initial episode, as well as during any recurrence (14,15,30,31). ADAMTS-13 activity is normal in these patients following recovery from either single or recurrent episodes. IgG antibodies (presumably autoantibodies) that inhibit plasma ADAMTS-13 activity can be detected during episodes in 48–94% of acquired TTP patients (18,30–32,55). These findings suggest that a transient, or intermittently recurrent, defect of immune regulation causes the transient, or recurrent, severe ADAMTS-13 deficiency in many patients with acquired TTP. Antibodies that inhibit plasma ADAMTS-13 have also been demonstrated in the few patients studied with ticlopidine or clopidogrel-associated TTP (14,15), or TTP related to pregnancy (18).

FIG. 5. (A) In normal individuals, ADAMTS-13 enzyme molecules from the plasma attach to, and then cleave, the ULVWF multimers that are secreted in long "strings" from stimulated endothelial cells. (B) The ULVWF multimeric strings may be anchored in the endothelial cell membrane to P-selectin molecules secreted concurrently with the ULVWF multimers from Weibel-Palade bodies. The ADAMTS-13 molecules attach to exposed A3 domains, and then cleave Tyr 842–843 Met peptide bonds in the adjacent A2 domains, of ULVWF monomeric subunits. Each ADAMTS-13 molecule may attach via its C-terminal CUB domain(s) to the exposed A3 domains. The smaller VWF forms that circulate after cleavage do not induce the adhesion and aggregation of platelets during normal blood flow. (C) Absent or severely reduced activity of ADAMTS-13 in patients with TTP prevents the timely cleavage of the "sticky" ULVWF multimers as they are secreted by endothelial cells. Uncleaved ULVWF multimers induce the adhesion and aggregation of platelets in flowing blood. Either congenital deficiencies of ADAMTS-13 activity (caused by ADAMTS-13 gene mutations) or acquired inhibition of ADAMTS-13 (caused by autoantibodies) result in TTP.

It is not yet known if there is a transient, severe defect of metalloprotease production or survival in those patients with acquired TTP who do not have detectable autoantibodies against ADAMTS-13. The failure to detect autoantibodies in some of these patients may simply reflect the variable sensitivity of test systems in current use.

Plasma ADAMTS-13 activity in healthy adults ranges from about 50–178%. Activity is often reduced below normal in liver disease, disseminated malignancies (56), chronic metabolic and inflammatory conditions, pregnancy, and in newborns (57). With the exception of those peri-partum women who develop TTP (18,52), the ADAMTS-13 activity in these conditions is not reduced to the extremely low values (<5% of normal) found in most patients with familial or acquired TTP.

21ST CENTURY THERAPY

The partial sequence of ADAMTS-13 has been determined, and the enzyme has been partially purified from normal human plasma fractions (33,43). Recombinant ADAMTS-13 has also been prepared (44). As a consequence, purified or recombinant ADAMTS-13 is a practical possibility for therapeutic use in TTP. A plasma level of ADAMTS-13 of only about 5% of normal is sufficient to prevent or truncate TTP episodes in most patients (3,51,58,59). Gene therapy, consequently, may eventually be capable of providing more lasting remissions in children with familial, chronic relapsing TTP.

Some patients with acquired TTP have high titers (60) or high affinity antibodies that inhibit plasma ADAMTS-13 activity, and do not respond adequately to plasma exchange. It may be possible to suppress the production of autoantibodies against ADAMTS-13 in these individuals using glucocorticoids (9) or (in dire circumstances) splenectomy (61,62). Recent anecdotal reports suggest that rituximab, the monoclonal antibody against CD20 on B-lymphocytes, is a promising new approach to ADAMTS-13 autoantibody control in acquired TTP patients (63–66).

CHALLENGES

A type of thrombotic microangiopathy with clinical similarities to TTP occurs in some patients weeks to months after their exposure to several types of therapy. These include: mitomycin C; inhibitors of the Ca^{2+}-activated phosphatase, calcineurin [cyclosporine or tacrolimus (FK 506)]; quinine; combinations of chemotherapeutic agents; total-body irradiation; or allogeneic bone marrow, kidney, liver, heart, or lung transplantation (67–70). The microvascular thrombi in these

patients may be either predominantly renal or systemic. The pathophysiology is not yet known. The "classical" hemolytic-uremic syndrome (HUS), usually associated with ingestion of enterohemorrhagic *E. coli* that produce Shiga-like toxin, is a thrombotic microangiopathy with predominant renal dysfunction (3). Neither bone marrow transplantation-associated thrombotic microangiopathy nor "classical" HUS is usually associated with an absence or severely reduced level of plasma ADAMTS-13 activity using assays currently available (30,55,71–73). In contrast to the ADAMTS-13-deficient types of TTP, there is no consistently effective therapy for these other types of thrombotic microangiopathic disorders. "Breakthrough" observations, made by accident or design, are needed urgently.

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DISCUSSION

Kitchens, Gainesville: Great address from the leading scholar. Joel is much too modest with all the discoveries that he's made here. He's almost as smart as the lawyers I face daily defending doctors against this disease. Thank you for all the work, and literally unraveling this problem.

Moake, Houston: Bless you for those kind comments.

Atkinson, St. Louis: I was interested in your comments comparing TTP to HUS. Are you seeing much of an overlap or do you feel that these are almost always distinct syndromes? What are your thoughts in this area?

Moake: Almost all the HUS patients do not have extremely low plasma levels, that is, less than 5% of ADAMTS-13. HUS is a different disorder. The experiments that are ongoing and promising suggest that in classical HUS Shiga toxin-1 may cause some disruption of the renal endothelial cell surfaces and, consequently, interfere with the kinetics of ADAMTS-13 interaction with unusually large VWF multimers emerging from those cells. The plasma level of ADAMTS-13 is not reduced, but the actual interaction of the enzyme near the surface of the Shiga toxin-altered endothelial cell may be abnormal. Therefore, there is a delayed cleavage of the unusually large VWF multimers secreted by renal endothelial cells that may initiate (on top of the endothelial cells) platelet adhesion and subsequent aggregation and fibrin polymer formation characteristic of HUS.

Antman, Boston: We carry around in the Cardiology community the notion that clopidogrel is associated with a lower frequency of TTP than ticlopidine. First of all I wanted to know if that is really correct?

Moake: Yes it is.

Antman: Any idea about the mechanisms how drugs produce TTP?

Moake: No, but here's a moment of speculation. These patients have autoantibodies against the ADAMTS-13, perhaps as an autoimmune escape associated with the administration of either ticlopidine or Plavix. Plavix is much less commonly associated with TTP, as you mentioned. The mechanism may be similar to Aldomet that induces an escape of IgG autoantibodies directed against Rh components in red cell membranes and causes Coombs' test positivity and occasional autoimmune hemolytic anemia. I'm not certain that this analogy is exact, but I think there's some sort of perturbation of the immune system and a few people taking these drugs have an escape of IgG autoantibody formation. One or more epitopes on ADMTS-13 are the target. It's an important question, and I wish I were clever enough to design experiments to define the mechanism precisely.

Schrier, Colorado: We had a patient recently in her first trimester of pregnancy who had an acquired TTP and whose platelet count responded to phasmasphoresis. Then, however, the platelets would decrease again. Eventually we had to do a splenectomy with a dramatic positive response. How often is splenectomy necessary to treat TTP?

Moake: About 20% of acquired "out of the blue" TTP patients either won't respond adequately to plasma exchange or will have too many or too frequent or too serious relapses. An agent that has come into use and is likely to be important is rituximab. A number of patients have responded well to rituximab therapy when they have had high titers of antibodies against ADAMTS-13 and have not responded adequately to glucocorticoids and splenectomy. So, although splenectomy is useful, it may actually be that soon glucocorticoids will be followed by retuximab and that only glucocorticoid/retuximab failures will be subjected to splenectomy. To date, however, only 10 to 20 acquired TTP patients have been reported to receive (and mostly respond) to rituximab. There are more patients who have responded, but have not been described in print.

Luke, Cincinnati: When will we get enzyme replacement or the possibility of gene therapy for the relapsing patients?

Moake: The enzyme has been partially, albeit considerably, purified. As ADAMTS-13 is purified, it becomes less stable than in plasma. There's a recombinant, active form of the enzyme, so it can be expected that recombinant ADAMTS-13 will be available before long.

Luke: Will it be used prophalatically in these patients?

Moake: Yes, especially in the familial patients. These are mostly children, along with the few adults who have had the good fortune of having been infused periodically with plasma for decades (often accidentally initially). Most familial TTP patients are infused with plasma about every three weeks. So, I expect that the recombinant enzyme will be used prophylactically in this familial TTP subset. The effect of each plasma infusion lasts much longer than the half-life of ADAMTS-13 activity in plasma, which is about 2–3 days. This may reflect the fact that the enzyme floats in plasma, but is really destined to go to endothelial cell surfaces. The half-life of the enzyme on endothelial cell surfaces is probably much longer. The familial TTP children typically have relapses every three weeks. They would be excellent candidates for gene therapy because only 5% or more of plasma ADAMTS-13 activity is required to be free of relapses. So whatever genetically-altered ADAMTS-13 producing cells can be propagated and injected (fibroblasts, for example) every six months to a year, may be of practical benefit.