Mechanisms of Disease

THROMBOTIC MICROANGIOPATHIES

JOEL L. MOAKE, M.D.

THE thrombotic microangiopathies are microvascular occlusive disorders characterized by systemic or intrarenal aggregation of platelets, thrombocytopenia, and mechanical injury to erythrocytes. In thrombotic thrombocytopenic purpura, systemic microvascular aggregation of platelets causes ischemia in the brain and other organs. In the hemolytic–uremic syndrome, platelet–fibrin thrombi occlude predominantly the renal circulation. Thrombotic thrombocytopenic purpura was initially described by Moschcowitz in 1924,[1] and the hemolytic–uremic syndrome by Gasser et al.[2] in 1955. Both disorders remained mysterious until the 1980s. In 1982, "unusually large" multimers of von Willebrand factor released from endothelial cells were found to accumulate in the plasma of patients with chronic relapsing thrombotic thrombocytopenic purpura,[3] and a failure to process these multimers was proposed to explain the disorder. In 1985, Karmali et al.[4] discovered a link between the hemolytic–uremic syndrome and enteric infections with *Escherichia coli* that produce Shiga toxin. These clues provoked a deluge of investigations that have elucidated the mechanisms of thrombotic thrombocytopenic purpura and the hemolytic–uremic syndrome. Table 1 summarizes the thrombotic microangiopathies that will be discussed in this review.

CLINICAL PRESENTATIONS

Thrombotic microangiopathies are characterized by thrombocytopenia (with increased numbers of marrow megakaryocytes), fragmentation of erythrocytes, and extremely elevated serum levels of lactate dehydrogenase. The severity of these abnormalities reflects the extent of the microvascular aggregation of platelets. Fragmented erythrocytes (schistocytes, or helmet cells) are probably produced as blood flows through turbulent areas of the microcirculation that are partially occluded by platelet aggregates. This process causes microangiopathic hemolytic anemia. The serum lactate dehydrogenase is largely derived from ischemic or necrotic tissue cells rather than from lysed red cells.[5]

In patients with thrombotic thrombocytopenic purpura, the systemic clumping of platelets mediated by unusually large multimers of von Willebrand factor often results in platelet counts below 20,000 per cubic millimeter during an acute episode. Ischemia of the brain or gastrointestinal tract is common, and renal dysfunction may occur. A pentad of signs and symptoms has been associated with thrombotic thrombocytopenic purpura: thrombocytopenia, microangiopathic hemolytic anemia, neurologic abnormalities, renal failure, and fever.[6] In actual practice, however, the triad of thrombocytopenia, schistocytosis, and elevated lactate dehydrogenase levels is often sufficient to suggest the disorder. If severe renal failure is the predominant feature at presentation, then the disorder is often considered to be the hemolytic–uremic syndrome.[7,8] The clinical distinction between thrombotic thrombocytopenic purpura and the hemolytic–uremic syndrome is not always clear-cut, however.[4,9-11] Renal abnormalities in patients who have been given a diagnosis of thrombotic thrombocytopenic purpura and extrarenal manifestations in some patients who have received a diagnosis of hemolytic–uremic syndrome can obscure clinical boundaries.[12-14] Nevertheless, it is often possible to recognize specific types of thrombotic microangiopathies.

Familial thrombotic thrombocytopenic purpura is rare. It may appear initially in infancy or childhood[15,16] and then recur at regular intervals of about three weeks (referred to as chronic relapsing thrombotic thrombocytopenic purpura). In some patients, however, a familial predisposition to the disorder may not be clinically evident for years.[17] Acquired idiopathic thrombotic thrombocytopenic purpura occurs in adults and older children and is usually characterized by a single acute episode. The episodes recur at irregular intervals in 11 to 36 percent of patients.[12,13,18,19] Thrombotic thrombocytopenic purpura develops within a few weeks after the initiation of therapy in a small fraction of patients with arterial thrombosis who receive ticlopidine, an inhibitor of one of the platelet adenosine diphosphate (ADP) receptors,[20,21] and an even smaller fraction of those who receive the structurally similar agent clopidogrel.[22] The disorder also occurs occasionally during pregnancy (especially the last trimester) or in the postpartum period.[23]

From Baylor College of Medicine and Rice University — both in Houston. Address reprint requests to Dr. Moake at the Hematology–Oncology Section, Methodist Hospital, MS 902, Main Bldg., 6565 Fannin, Houston, TX 77030, or at jmoake@rice.edu.

TABLE 1. THROMBOTIC MICROANGIOPATHIES.

TYPE OF MICROANGIOPATHY	CAUSE	CLINICAL PRESENTATION
Systemic platelet thrombi	Failure to degrade unusually large multi-mers of von Willebrand factor	Thrombotic thrombocytopenic purpura
Predominantly renal platelet–fibrin thrombi	Exposure to Shiga toxin	Classic, childhood, or *Escherichia coli*–associated hemolytic–uremic syndrome
	Defect in plasma factor H	Familial (or recurrent) hemolytic–uremic syndrome
Renal or systemic thrombi	Transplantation or drugs (mitomycin, cyclosporine, tacrolimus, quinine)	Hemolytic–uremic syndrome or thrombotic thrombocytopenic purpura

The hemolytic–uremic syndrome usually occurs as a single episode, often preceded by gastroenteritis caused by cytotoxin-producing gram-negative bacteria, most commonly *E. coli* O157:H7.[4] A recurrent illness resembling the hemolytic–uremic syndrome may be caused by defective production of the complement control protein factor H.[11,24,25]

A type of thrombotic microangiopathy with either predominantly renal or systemic thrombi occurs in some patients who receive mitomycin, cyclosporine, tacrolimus, quinine, a marrow or organ transplant, total-body irradiation, or combinations of chemotherapeutic agents.[14]

PATHOPHYSIOLOGY

Thrombotic Thrombocytopenic Purpura

Microvascular thrombi occur in most organs in patients with thrombotic thrombocytopenic purpura and consist of platelet aggregates with little or no fibrin; there is no perivascular inflammation or overt endothelial-cell damage.[26] The platelet thrombi contain abundant von Willebrand factor antigen but no fibrinogen (or fibrin), whereas the platelet thrombi in disseminated intravascular coagulation contain fibrin but not von Willebrand factor.[27] Flow-cytometric studies demonstrate that levels of von Willebrand factor antigen attached to single platelets in whole-blood samples are higher during episodes of thrombotic thrombocytopenic purpura than during periods of recovery or remission.[28] The results of clotting studies during these episodes are usually normal.

Monomers of von Willebrand factor (280 kD) are linked by disulfide bonds to form multimers with various molecular masses that range into the millions of daltons.[29] Multimers of von Willebrand factor are constructed within megakaryocytes and endothelial cells and stored within platelet α-granules and endothelial-cell Weibel–Palade bodies. Most multimers in plasma come from endothelial cells. Both endothelial cells and platelets produce multimers of von Willebrand factor that are larger than those in normal plasma.[3] These unusually large multimers bind more efficiently than the largest plasma multimers to the glycoprotein Ibα component of platelet glycoprotein Ib/IX/V receptors for von Willebrand factor.[30,31] This is probably because the binding sites for glycoprotein Ibα in monomeric subunits of von Willebrand factor are more effectively exposed in unusually large multimers than in the smaller forms that are normally in circulation.[31] The initial attachment of only a small quantity of unusually large multimers of von Willebrand factor to glycoprotein Ibα, and subsequently to ADP-activated platelet glycoprotein IIb/IIIa complexes,[30,32] induces platelet aggregation in vitro in the presence of increased fluid shear stress.

A von Willebrand factor–cleaving metalloprotease in plasma normally prevents the entrance into the circulation (or persistence) of unusually large multimers of von Willebrand factor. This enzyme degrades the multimers by cleaving peptide bonds in monomeric subunits of von Willebrand factor at position 842–843 (between tyrosine and methionine).[33,34] The metalloprotease is referred to as ADAMTS 13 (a disintegrin and metalloprotease, with thrombospondin-1–like domains), a member of a family of zinc- and calcium-dependent proteases. ADAMTS 13 has an arginine–glycine–aspartate (RGD) sequence, its gene is on chromosome 9q34, and it is produced predominantly by hepatocytes.[33-39]

Unusually large multimers of von Willebrand factor are probably cleaved by ADAMTS 13 directly on the surface of endothelial cells (Fig. 1).[40] The thrombospondin-1–like domains in ADAMTS 13 may bind the enzyme to thrombospondin receptors on the surface of endothelial cells. Partial unfolding of emerging unusually large multimers as a result of fluid shear stress[39,40] may increase the efficiency of cleavage by ADAMTS 13 (Fig. 2).[41,42] Table 2 summarizes the relation between defects in ADAMTS 13 and the various clinical presentations of thrombotic thrombocytopenic purpura.

Normal washed
platelets in
buffer

Normal washed
platelets in
normal plasma

Normal washed
platelets in
plasma from
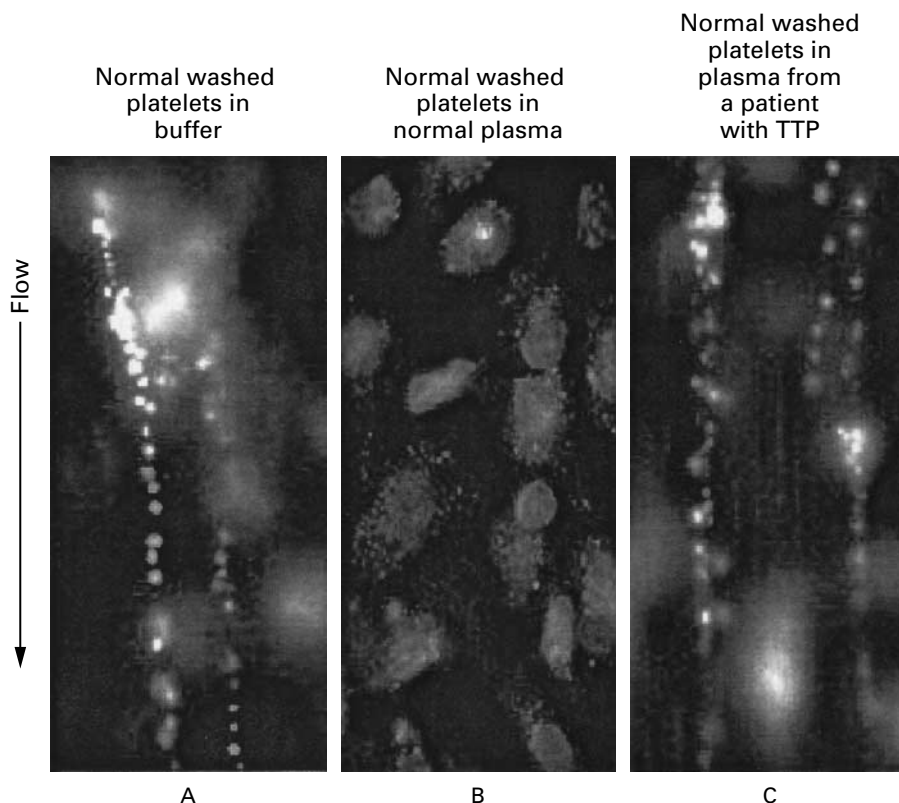a patient
with TTP

Flow

A

B

C

Figure 1. Cleavage of Unusually Large Multimers of von Willebrand Factor on Endothelial Cells by the von Willebrand Factor–Cleaving Metalloprotease, ADAMTS 13.

In vivo, the von Willebrand factor–cleaving metalloprotease, ADAMTS 13, may cleave unusually large multimers of von Willebrand factor as they exit endothelial cells. When washed, fluorescently labeled platelets from a normal subject are suspended in buffer, they adhere by means of glycoprotein Ibα to long strings of these unusually large multimers that have been secreted from, and remain attached to, histamine-stimulated endothelial cells (Panel A).[40] The phenomenon occurs under conditions of both venous and arterial flow (2 to 60 dyn per square centimeter). In the presence of normal plasma containing partially purified von Willebrand factor–cleaving metalloprotease, the unusually large multimers of von Willebrand factor are cleaved within seconds to a few minutes as they are secreted by endothelial cells (Panel B). The secreted, attached unusually large multimers on endothelial cells are not cleaved during more than 10 minutes of flow in the presence of plasma from a patient with thrombotic thrombocytopenic purpura (TTP), indicating that there is no metalloprotease activity (Panel C).

In most patients with familial or acquired types of thrombotic thrombocytopenic purpura, plasma ADAMTS 13 activity is less than 5 percent of normal.[16,21,22,43-45] A severe deficiency of ADAMTS 13 activity in plasma from patients with familial or acquired thrombotic thrombocytopenic purpura correlates with deficient ADAMTS 13 activity on the surface of endothelial cells (Fig. 1). As a consequence, the unusually large multimers of von Willebrand factor are not cleaved after they are secreted from endothelial cells and instead remain anchored to the cells in long strings (Fig. 1 and 2).[40] Passing platelets adhere by means of their glycoprotein Ibα receptors to these long multimers. (Platelets do not adhere to the smaller von Willebrand factor forms produced by cleavage of unusually large multimers.[29]) Many additional platelets subsequently aggregate by means of their activated glycoprotein IIb/IIIa complexes onto the unusually large multimeric strings. As a result, large, potentially occlusive platelet thrombi are formed (Fig. 2). An additional event — intense stimulation of secretion of unusually large multimers by endothelial cells — may provoke episodes of thrombotic thrombocytopenic purpura in some patients.

Patients with familial thrombotic thrombocytopenic purpura frequently have unusually large multimers of von Willebrand factor in their plasma.[3,46] Their plasma ADAMTS 13 activity is zero (or barely detectable)[16,43,47] as a consequence of homozygous (or double heterozygous) mutations in each of the two 9q34
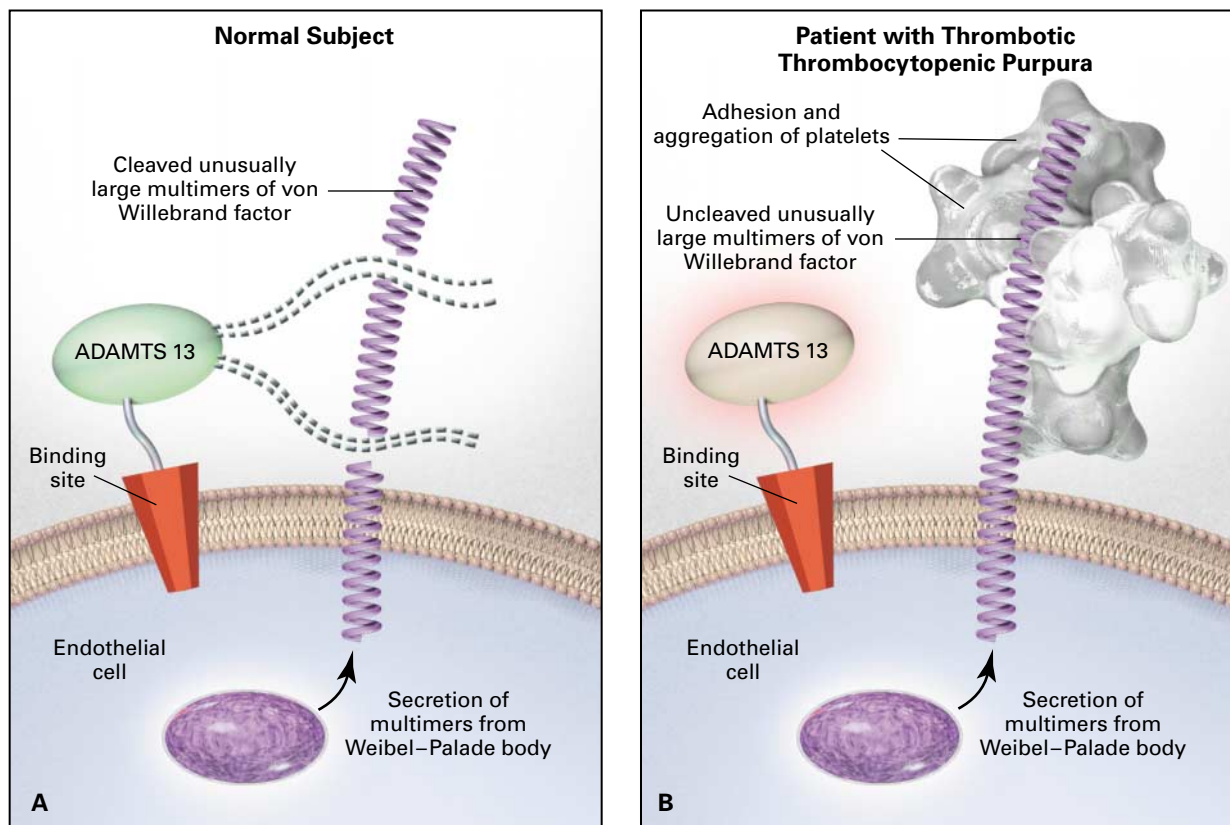
Figure 2. Proposed Relation among the Absence of ADAMTS 13 Activity in Vivo, Excessive Adhesion and Aggregation of Platelets, and Thrombotic Thrombocytopenic Purpura.

In Panel A, in normal subjects, ADAMTS 13 (von Willebrand factor–cleaving metalloprotease) molecules attach to binding sites on endothelial-cell surfaces and cleave unusually large multimers of von Willebrand factor as they are secreted by stimulated endothelial cells. The smaller von Willebrand factor forms that circulate after cleavage do not induce the adhesion and aggregation of platelets during normal blood flow. ADAMTS 13 may use one of its thrombospondin-1–like domains or its arginine–glycine–aspartate (RGD) sequence to attach to the surface of endothelial cells. In Panel B, absent or severely reduced activity of ADAMTS 13 in patients with thrombotic thrombocytopenic purpura prevents timely cleavage of unusually large multimers of von Willebrand factor as they are secreted by endothelial cells. The uncleaved multimers induce the adhesion and aggregation of platelets in flowing blood. A congenital deficiency of ADAMTS 13 activity, or an acquired defect of ADAMTS 13 (such as that caused by autoantibodies or by a change in the production or survival of the protein) can lead to thrombotic thrombocytopenic purpura. Interference with the attachment of ADAMTS 13 to endothelial cells in vivo (for example, as a result of ADAMTS 13–receptor blockade by other types of autoantibodies) may also cause thrombotic thrombocytopenic purpura in patients with normal ADAMTS 13 activity in plasma.

genes that encode ADAMTS 13.[37] In most patients with a severe familial deficiency of ADAMTS 13 activity, episodes of thrombotic thrombocytopenic purpura begin in infancy or childhood. In others, however, the disease does not develop for years (perhaps during a first pregnancy), and a few may never have an episode. In the occasional patient with severe familial deficiency of plasma ADAMTS 13 activity who either has a delayed onset of thrombotic thrombocytopenic purpura or has not had any episodes, the physiologic ADAMTS 13 activity on the surface of endothelial cells may exceed estimates of enzyme activity obtained with in vitro fluid-phase plasma assays.

During an episode of acquired idiopathic thrombotic thrombocytopenic purpura or any recurrence, patients usually have undetectable or barely detectable plasma levels of ADAMTS 13.[21,43,44] The activity is normal after recovery. IgG antibodies (presumably autoantibodies) that inhibit enzyme activity in plasma are found in 48 to 80 percent of these patients,[43-45,48] suggesting the presence of a transient (or intermittent) defect of immune regulation. Antibodies that inhibit the plasma metalloprotease have also been demonstrated in a few patients with thrombotic thrombocytopenic purpura associated with ticlopidine (or clopidogrel) use.[21,22] It is not known whether there is a

TABLE 2. RELATION BETWEEN DEFECTS IN PLASMA VON WILLEBRAND FACTOR−CLEAVING METALLOPROTEASE, ADAMTS 13, AND THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP).

DEFECT	CLINICAL PRESENTATION
ADAMTS 13 plasma activity <5% of normal	
Mutations in the gene for ADAMTS 13	Familial TTP, chronic relapsing TTP
Disease presentation in infancy or childhood	
Disease presentation later in life	
Autoantibodies against ADAMTS 13	Acquired idiopathic TTP
Transient	Single-episode TTP
Recurrent	Recurrent (intermittent) TTP
Ticlopidine-associated*	Ticlopidine-associated TTP
Transient defect in production or survival of ADAMTS 13†	Acquired idiopathic TTP
Normal ADAMTS 13 activity in plasma with defective attachment of ADAMTS 13 to endothelial cells†	Familial and acquired TTP†

*Cases associated with clopidogrel, which is structurally similar to ticlopidine, have been reported.

†This possibility has yet to be proved.

transient, severe defect of metalloprotease production or survival in patients with acquired idiopathic thrombotic thrombocytopenic purpura who do not have detectable autoantibodies against ADAMTS 13 with the use of in vitro assays.

Plasma ADAMTS 13 activity in healthy adults ranges from about 50 to 178 percent of normal. The level of activity is often below normal in patients with liver disease, disseminated cancers,[49] and chronic metabolic and inflammatory conditions; pregnant women; and newborns.[50] These moderately reduced levels are in contrast to the extremely low levels (less than 5 percent of normal values) in most patients with episodes of familial or acquired thrombotic thrombocytopenic purpura.[16,21,22,43-45,47,48]

Some patients with acquired idiopathic thrombotic thrombocytopenic purpura have unusually large multimers of von Willebrand factor in their plasma in the absence of severely reduced levels of plasma metalloprotease activity in vitro.[48,51] Another mechanism must explain the inadequate in vivo function of ADAMTS 13 in these patients. They might, for example, produce autoantibodies that prevent the attachment of ADAMTS 13 to endothelial-cell−binding sites without interfering with the active site of the metalloprotease. It may be relevant that autoantibodies against glycoprotein IV (CD36),[52] a cell-surface thrombospondin receptor, appear in the plasma of some patients during episodes of thrombotic thrombocytopenic purpura. Whether these antibodies interfere with the attachment of ADAMTS 13, through one of its thrombospondin-1−like domains, to CD36 thrombospondin receptors on the surface of endothelial cells is unknown.

The Hemolytic−Uremic Syndrome

The hemolytic−uremic syndrome occurs in 9 to 30 percent of infected children about a week after an episode of bloody diarrhea caused by *E. coli* O157: H7.[4,10,53] Infections with other *E. coli* serotypes, *Shigella dysenteriae,* and (occasionally) other microbes also cause the hemolytic−uremic syndrome in children and adults. In Buenos Aires, Argentina, and Calgary, Canada, enterohemorrhagic *E. coli* infections are endemic and the hemolytic−uremic syndrome is a common cause of acute renal failure in children.[10]

Shiga toxin is a 70-kD protein exotoxin encoded by *S. dysenteriae* DNA,[54] whereas Shiga toxins 1 and 2 are encoded by bacteriophage DNA, which can be present in several *E. coli* serotypes.[54] Shiga toxin consists of one A subunit (33 kD) and five B, or binding, subunits (7.7 kD each) (Fig. 3A).[54] Each B subunit binds with high affinity to terminal galactose $\alpha1,4\beta$ galactose disaccharides in globotriaosylceramide receptors[54,55] (Fig. 3A) in the membranes of glomerular, colonic, and cerebral epithelial or microvascular endothelial cells; renal mesangial and tubular cells; and monocytes and platelets.[54,56-59]

Strains of *S. dysenteriae* that are capable of producing Shiga toxin and *E. coli* serotypes that produce Shiga toxin 1 or 2 can contaminate meat, milk, cheese, and other types of insufficiently cooked or pasteurized food.[10,60] Cattle are a major reservoir of *E. coli* O157:H7, but they remain well because their vascular endothelial cells lack the globotriaosylceramide receptors necessary to bind Shiga toxins 1 and 2.[61]

In humans, the enterohemorrhagic bacteria adhere to mucosal epithelial cells of the colon and invade, replicate, and destroy the cells. The Shiga exotoxins
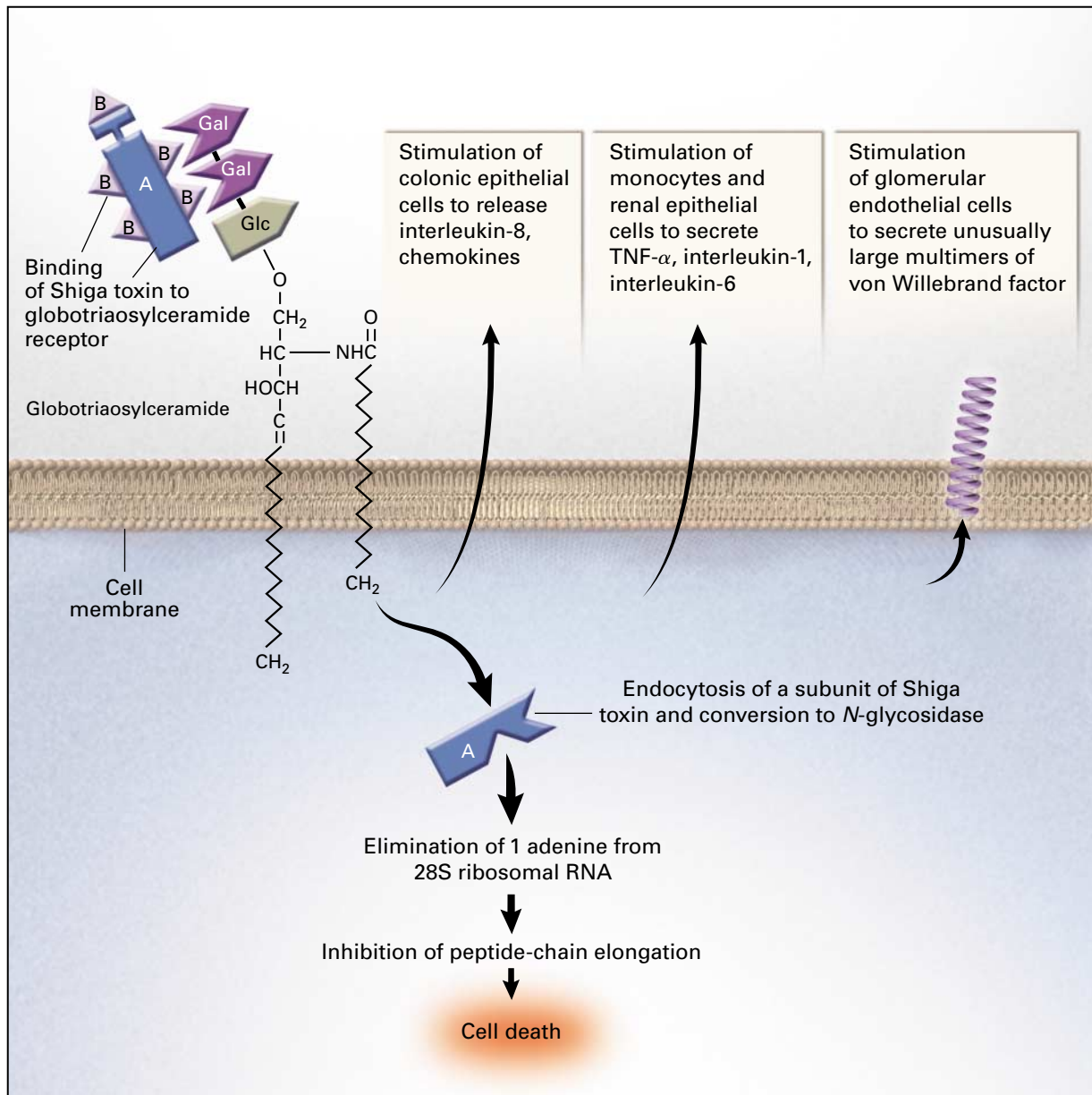
Figure 3. Role of Shiga Toxin, Cytokines, Unusually Large Multimers of von Willebrand Factor, and Cellular Injury.

The B subunits of polymeric Shiga toxin molecules attach to specific disaccharides of globotriaosylceramide receptors in the membranes of colonic epithelial cells, monocytes, platelets, glomerular and tubular epithelial cells, renal mesangial cells, and glomerular and cerebrovascular endothelial cells. This action stimulates epithelial cells and monocytes to secrete cytokines and chemokines, stimulates endothelial cells to secrete unusually large multimers of von Willebrand factor, and activates platelets. The binding of Shiga toxin to globotriaosylceramide receptors may also increase tissue factor on endothelial cells and epithelial-cell surfaces. Once the A subunit of Shiga toxin is internalized, it is converted to a glycosidase whose actions ultimately result in cell death. The globotriaosylceramide receptors associate with cholesterol and ganglioside to form lipid rafts that float in plasma membranes. TNF-α denotes tumor necrosis factor α.

(probably together with bacterial-endotoxin lipopolysaccharides)[10,54] damage the underlying tissue and vasculature and cause bloody diarrhea. The injury is potentiated by neutrophils, which are recruited into the damaged colon and activated by the cytokine interleukin-8 and the CXC chemokines: growth-related oncogenes α, β, and γ and epithelial-cell–derived neutrophil-activating peptide 78.[62,63] Interleukin-8 and these chemokines are secreted by colonic epithelial cells in response to binding of Shiga toxin 1 to globotriaosylceramide on the cell surface.

Shiga toxin from *S. dysenteriae* and Shiga toxins 1 and 2 from enterohemorrhagic *E. coli* then enter the intestinal circulation and travel in the plasma and on the surface of platelets or monocytes.[59,64] The toxins attach to molecules on glomerular capillary endothelial cells, mesangial cells, and glomerular and tubular epithelial cells.[65-68] Shiga toxin 1 (especially in association with bacterial-endotoxin lipopolysaccharide) stimulates the release of tumor necrosis factor α, interleukin-1, and interleukin-6 from monocytes and renal glomerular and tubular epithelial cells (Fig. 3).[64,68-71] These cytokines up-regulate the expression of globotriaosylceramide on renal endothelial cells and increase the binding of Shiga toxin 1.[54,72,73] Shiga toxin 1 initially induces the endothelial cells to secrete unusually large multimers of von Willebrand factor[14] and increases the exposure of vitronectin ($\alpha_v\beta_3$ integrin) receptors, P-selectin, and platelet endothelial-cell adhesion molecule 1 on cell surfaces.[72] In addition, Shiga toxin 1 binds to and activates platelets by means of globotriaosylceramide or globotriaosylceramide-like receptors.[59] These events may promote the adhesion and aggregation of platelets onto the unusually large multimers of von Willebrand factor protruding from endothelial-cell surfaces (Fig. 4A).

The hemolytic–uremic syndrome is not usually associated with the absence or severe reduction of plasma ADAMTS 13 activity.[44,48,74,75] If Shiga toxin 1 were to impair ADAMTS 13 activity on altered surfaces of glomerular endothelial cells, then the proteolysis of secreted unusually large multimers of von Willebrand factor might be delayed long enough to allow the binding of Shiga toxin 1–activated platelets (by means of glycoprotein Ibα and IIb/IIIa complexes)[72] to uncleaved multimers on the cell surface.

In kidney cells, the A subunit of Shiga toxin, Shiga toxin 1, and Shiga toxin 2 undergoes partial proteolysis and disulfide-bond reduction (Fig. 3). These processes generate an enzyme that cleaves an adenine from 28S ribosomal RNA,[54] inhibits the elongation of peptide chains and the synthesis of proteins, and causes cell death. The damage is potentiated by the monocytes and neutrophils that invade the glomeruli in response to the secreted interleukin-8 and the production of monocyte chemoattractant protein 1 by renal cells.[73,76] Interleukin-8–activated neutrophils release oxygen-derived free radicals, hydrogen peroxide, elastase, and other proteases that potentiate the damage to the kidneys.[77-79] In fact, neutrophilia increases the likelihood of irreversible renal injury.

The death and desquamation of endothelial cells in the kidney may also promote, by means of glycoprotein Ibα, the adhesion of platelets to unusually large multimers of von Willebrand factor in the subendothelium.[31,80] Subsequent binding of fibrinogen to activated platelet glycoprotein IIb/IIIa complexes induces the aggregation of platelets under conditions of high flow (high shear),[80] as occur in the glomerular microcirculation. (In contrast to the situation in thrombotic thrombocytopenic purpura, in the hemolytic–uremic syndrome von Willebrand factor antigen is not prominent within glomerular platelet–fibrin thrombi.[75]) Increased exposure of tissue factor and binding of activated factor VII to perturbed cell surfaces may be followed by the generation of thrombin and the formation of fibrin polymers in *E. coli*–associated hemolytic–uremic syndrome (Fig. 4B).[81]

Deficiency of Plasma Factor H

Familial hemolytic–uremic syndrome accounts for 5 to 10 percent of all cases of the disorder. The mortality rate (54 percent) is much higher than that in typical childhood hemolytic–uremic syndrome (3 to 5 percent). About half of survivors have relapses, and over one third require long-term dialysis.[82] Among patients with familial hemolytic–uremic syndrome who receive kidney allografts, 16 percent lose function in the engrafted kidney within one month.[83] Some (or most) of these patients have a deficiency or defect of complement factor H[24,82,84,85]; an area on chromosome 1q32 that overlaps the factor H gene segregates with the disorder.[24]

Factor H is a 150-kD plasma protein containing 20 short consensus repeats of 60 amino acids each.[86] Factor H normally protects host cells from accidental damage by the alternative complement pathway. C3bBb, the C3 convertase of the alternative pathway, amplifies the generation of C3b molecules on the surface of susceptible cells. Factor H regulates this process by displacing Bb from C3b, thereby exposing C3b to cleavage and inactivation by factor I (the C3b inactivator). A deficiency or dysfunction of factor H results in overactivation of C3, which may potentiate autoantibody-mediated or immune-complex–mediated glomerular injury.[82] The result may be damage and desquamation of glomerular endothelial cells, exposure of glomerular subendothelium, adhesion and aggregation of platelets, increased local tissue factor with factor VII binding and activation, and the generation of thrombin and the formation of fibrin polymers.
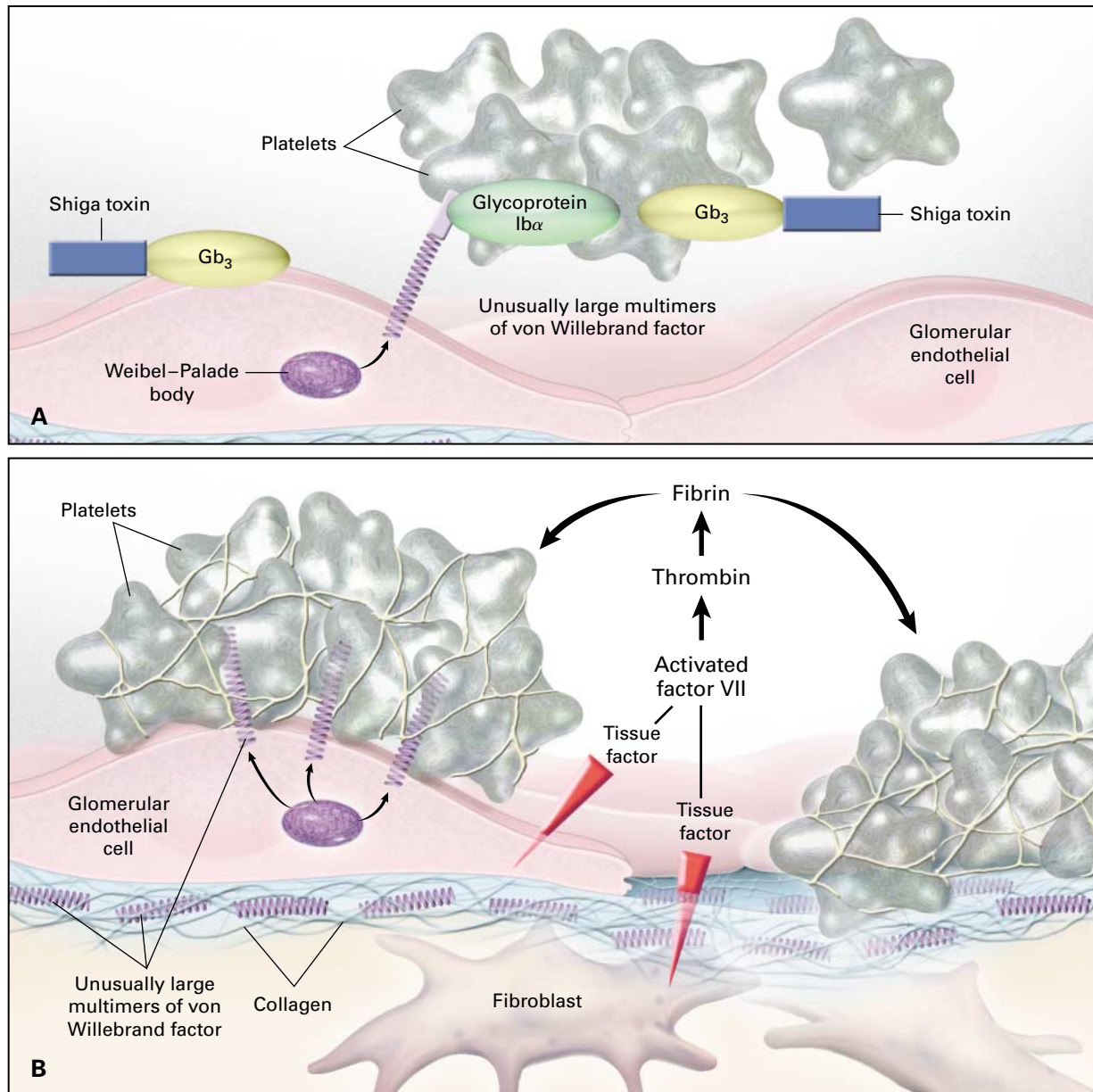
Figure 4. Proposed Mechanisms of Platelet–Fibrin Formation in the Hemolytic–Uremic Syndrome.

In Panel A, platelets activated by Shiga toxin may adhere by means of the glycoprotein Ibα components of glycoprotein Ib/IX/V complexes to unusually large multimers of von Willebrand factor that are secreted from toxin-stimulated renal endothelial cells. The adherence of platelets to endothelial cells is especially likely if the cleavage of multimers extruding from endothelial cells by von Willebrand factor–cleaving metalloprotease, ADAMTS 13, is impaired by the interactions of Shiga toxin with globotriaosylcer-amide (Gb$_3$) receptors on endothelial-cell surfaces. The activation of platelets that is mediated by Shiga toxin may contribute to the aggregation of additional platelets. In Panel B, endocytosis and activation of the A subunits of Shiga toxin may cause the death and desquamation of endothelial cells, exposing unusually large multimers of von Willebrand factor entwined with collagen in the sub-endothelium. Platelets from flowing blood in the renal microcirculation may then adhere and aggregate on the exposed multimers and collagen. Local exposure of tissue factor and binding and activation of factor VII may occur on fibroblasts, invading phagocytic cells, and injured renal endothelial and epithelial cells. These actions may, in turn, induce the activation of factors IX and X, cleavage of prothrombin to thrombin by the complex of activated factor X and activated factor V, and the thrombin-induced formation of fibrin polymers, thus potentiating renal microvascular thrombosis.

Point mutations, deletions, and frame shifts in the factor H gene have been identified in patients and their relatives. Most mutations occur in short-consensus-repeat number 20 of the factor H gene,[87,88] which includes one of the domains that enables factor H to attach to C3b.[86-88] Both autosomal recessive and autosomal dominant forms of inheritance have been described.[86,88,89] Recessive inheritance is associated with factor H levels that are 10 to 50 percent of normal as a result of aberrant protein folding and decreased secretion. The serum C3 level is low, and the hemolytic–uremic syndrome develops at a young age.[88,90] Dominant inheritance is associated with a functionally abnormal factor H protein with a normal serum antigenic value, normal serum C3 levels, and delayed onset of the hemolytic–uremic syndrome, which may be precipitated by infection or pregnancy.[88,89]

Renal or Systemic Thrombotic Microangiopathies of Unknown Causes

Thrombotic microangiopathy has been associated with mitomycin, cyclosporine, tacrolimus, combinations of chemotherapeutic agents, and total-body irradiation weeks or months after exposure to these agents.[71] Thrombi may be predominantly renal or systemic and have been reported after allogeneic bone marrow, kidney, liver, heart, or lung transplantation.[91,92] In the type of thrombotic microangiopathy that is associated with bone marrow transplantation, the activity of ADAMTS 13 in plasma is not usually reduced.[74] The mechanism of these microangiopathies is unknown.

In quinine-induced immune thrombocytopenia, patients produce antibodies against epitopes of platelet glycoprotein Ib/IX/V or IIb/IIIa complexes that have been antigenically altered by the attachment of quinine. In some of these patients, thrombotic microangiopathy also develops,[93,94] possibly because the antibodies cross-react with quinine-altered glycoprotein IIIa molecules on endothelial-cell membranes.

THERAPY

Thrombotic Thrombocytopenic Purpura

Infants or young children with familial thrombotic thrombocytopenic purpura produce a functionally defective ADAMTS 13.[16,37,47] Their episodes of thrombotic thrombocytopenic purpura are reversed or prevented by the infusion of platelet-poor fresh-frozen plasma, cryoprecipitate-poor plasma (cryosupernatant), or plasma that has been treated with a mixture of an organic solvent and detergent.[15] Plasmapheresis is not required.[15] Fresh-frozen plasma, cryosupernatant, and plasma treated with a mixture of solvent and detergent all contain the active metalloprotease. It is not known why infusion of the metalloprotease is required only about every three weeks. The plasma half-

life of the infused enzyme is about two days,[47] and the half-life of the enzyme once it is attached to the endothelial-cell surface may be even longer.

The sequence of ADAMTS 13 has been determined, and the enzyme has been partially purified from normal human plasma.[35,36] These advances may make purified metalloprotease products available for use in thrombotic thrombocytopenic purpura. Since a plasma level of only about 5 percent is sufficient to prevent or shorten episodes of thrombotic thrombocytopenic purpura,[95,96] gene therapy may induce lasting remissions in children with the chronic relapsing form of the disease.

Adults and older children with acquired acute idiopathic thrombotic thrombocytopenic purpura require daily plasma exchange.[13,18] Plasma exchange is the combination of plasmapheresis (which may remove unusually large multimers of von Willebrand factor and autoantibodies against ADAMTS 13) and infusion of fresh-frozen plasma or cryosupernatant (containing additional metalloprotease). Plasma exchange allows about 90 percent of these patients to survive an episode of thrombotic thrombocytopenic purpura,[12,13] usually without permanent organ damage.[12]

Some patients with acquired acute idiopathic thrombotic thrombocytopenic purpura and high titers of antibodies against ADAMTS 13[97] do not respond to plasma exchange alone. It may be possible to interfere with autoantibody production through treatment with glucocorticoids[12] or splenectomy[98,99] or to depolymerize platelet microtubules and alter exposure of surface receptors by infusing vincristine.[100] Rituximab, the monoclonal antibody against CD20 on B-lymphocytes, is under investigation. In the absence of life-threatening hemorrhage or intracranial bleeding, it is prudent to avoid platelet transfusions, which can exacerbate microvascular thrombosis.[12,26] Aspirin may provoke hemorrhagic complications in patients with severe thrombocytopenia.[101]

The Hemolytic–Uremic Syndrome

In mildly affected children with the hemolytic–uremic syndrome who have had oligoanuria for less than 24 hours, appropriate management of fluid and electrolyte levels is usually sufficient. Otherwise, the duration of anuria and attendant dialysis support correlates inversely with the likelihood of full recovery. Acute renal failure is often more severe in adults. Ultimately, care for end-stage renal disease may be required.

Plasma infusion or exchange has been tried, with equivocal results.[102,103] Even the infusion of normal fresh-frozen plasma (containing factor H) in patients with familial hemolytic–uremic syndrome has not succeeded in preventing relapses or progressive renal disease.[24,25,84,85] Purified or recombinant factor H may

eventually be developed for use in patients with a deficiency of factor H. Plasma adsorption over staphylococcal protein A columns has been reported to be useful in cases of thrombotic microangiopathy due to mitomycin.[104]

Antimotility agents increase the risk of the hemolytic–uremic syndrome after infection with *E. coli* O157:H7. Antimicrobial agents increase the release of Shiga toxin from *E. coli* O157:H7,[105] and antibiotic therapy may thus paradoxically increase the risk of the hemolytic–uremic syndrome in infected children. Normal persons generate neutralizing antibodies against Shiga toxin in response to infection with toxin-producing microbes,[10] and the intranasal immunization of mice with the B subunit of *E. coli* Shiga toxin 1 elicits antibodies that neutralize the exotoxin.[106] These observations hold promise for the development of a vaccine against *E. coli* O157:H7. It is not known whether anticoagulant therapy is useful or safe.[81]

Overview of Therapy

Microvascular aggregation of platelets occurs in both thrombotic thrombocytopenic purpura and the hemolytic–uremic syndrome. If the aggregation of platelets is systemic, and especially if the central nervous system is involved, the disorder is usually called thrombotic thrombocytopenic purpura. If platelet aggregation is predominantly confined to the renal circulation, the hemolytic–uremic syndrome is often diagnosed. Severe renal involvement in a patient with a diagnosis of thrombotic thrombocytopenic purpura (renal abnormalities occur in 50 to 75 percent of episodes)[12,13] or extrarenal manifestations in a patient with a diagnosis of the hemolytic–uremic syndrome may erase clinical distinctions between the two entities.[12,13,107]

In most patients with a diagnosis of thrombotic thrombocytopenic purpura, ADAMTS 13 activity, measured on a pretreatment citrated plasma sample, is 0 to 5 percent of normal.[16,21,22,43-45,47,75,97] In contrast, plasma ADAMTS 13 activity is not as severely reduced (or is normal) in most patients considered clinically to have the hemolytic–uremic syndrome or other thrombotic microangiopathies.[43,48,50,75] The use of citrated plasma (or endothelial-cell–based plasma) ADAMTS 13 assays may eventually allow most patients with thrombotic thrombocytopenic purpura to be identified precisely. Rapid assays of the metalloprotease are not generally available and so are not yet capable of influencing emergency clinical decisions. In addition, the relation between pretreatment values obtained with the use of various evolving ADAMTS 13 testing methods and the clinical responsiveness to plasma exchange awaits further study. Currently, an adult patient who has an acquired syndrome that could be either thrombotic thrombocytopenic

purpura or the hemolytic–uremic syndrome should be presumed to have thrombotic thrombocytopenic purpura, and plasma exchange should be initiated as soon as possible.[18,107]

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