Thrombotic Thrombocytopenic Purpura in Humans and Mice

Karl C. Desch, David G. Motto

Abstract—Thrombotic thrombocytopenic purpura (TTP) is a disorder of blood coagulation that presents classically with the pentad of fever, thrombocytopenia, microangiopathic hemolytic anemia, renal dysfunction and mental status changes. However, the clinical presentation can be quite variable making the diagnosis difficult in many cases. “Hyaline” microthrombi composed primarily of platelets and Von Willebrand Factor (VWF) are found in the small vessels of affected organs and represent the pathological hallmark of the disease. The accompanying tissue ischemia is thought to explain the clinical TTP signs and symptoms. Pathogenesis of TTP has been linked to dysfunction of ADAMTS13, a metalloprotease whose only known substrate is VWF. Interestingly, further investigation into the natural history of TTP has demonstrated that ADAMTS13 deficiency likely is necessary, but not sufficient for the development of this disease, suggesting that additional genetic and/or environmental factors are required for TTP pathogenesis. Recently, a mouse model of TTP was established that recapitulates many of the key clinical features of this disease, including the requirement for further genetic and environmental factors in addition to ADAMTS13 deficiency. Therefore, in addition to being useful for the direct study of disease pathophysiology in vivo, this mouse model may also play a key role in elucidating some of the important environmental and genetic contributors to disease pathogenesis. Here we will briefly review TTP in humans, and then discuss recent information gained from the analysis of ADAMTS13-deficient mice. (Arterioscler Thromb Vasc Biol. 2007;27:0-0.)

Key Words: ●●●

Human TTP Pathogenesis
Historically, studies investigating TTP pathogenesis have focused on the roles played by endothelial cell dysfunction, platelet dysfunction,1,2 or vWF dysfunction.3 Recently, however, the role of abnormal vWF homeostasis has become the most widely supported hypothesis in TTP pathogenesis.4 vWF is an abundant plasma glycoprotein that provides the initial adhesive link between circulating blood platelets and sites of vascular injury. In addition, vWF acts as a carrier for coagulation Factor VIII in circulation.

The importance of vWF in maintaining hemostasis is illustrated clinically in patients with von Willebrand Disease (vWD). While type 1 vWD is associated with modestly decreased levels of circulating vWF with mild bruising and bleeding, type 3 vWD (severe vWF deficiency) can clinically mimic hemophilia A. vWF is synthesized in endothelial cells and megakaryocytes where it is processed from an initial propeptide monomer into considerably larger multimeric forms.5 Endothelial cell vWF is synthesized and released in both a constitutive and an inducible manner. vWF that is not released constitutively is stored in specialized organelles called Weibel-Palade bodies.6 On endothelial cell stimulation, the stored vWF is released into the circulation in a form termed ultra-large vWF (UL-vWF) which represents the most thrombogenic form of this molecule.7–10 On release into the circulation, UL-vWF is processed into smaller and less thrombogenic multimers by ADAMTS13, and therefore UL-vWF is not typically detected on multimer analysis of normal healthy human plasma.11

Identification of the crucial link between TTP pathogenesis and vWF metabolism was made in 1982 when Moake and colleagues demonstrated the presence of UL-vWF in the plasma of patients with familial TTP.12 These investigators hypothesized that deficiency of a vWF “depolymerase” was the underlying cause of TTP, and that UL-vWF played an important role in the formation of the platelet and vWF-rich clots characteristic of this disease. This vWF “depolymerase” was ultimately identified as the metalloprotease ADAMTS13.13–17 This enzyme cleaves vWF within the A2 domain between amino acid residues tyrosine 1605 and methionine 1606.18 In vitro activity of ADAMTS13 is dependent on factors such as fluid shear stress or by binding to platelets, the endothelium or possibly other...
molecules. Deficiency of ADAMTS13 can either be genetic (the rare Upshaw-Schulman syndrome) or more commonly, acquired, in the form of inhibitory anti-ADAMTS13 autoantibodies.

Clinical Characteristics of Human TTP

The annual incidence of acquired TTP in the United States is estimated to be 4 cases in 1,000,000 and appears to be increasing. Females are at greater risk with a female to male ratio of at least 3:2. Although patients with familial TTP can be successfully treated with plasma infusions to replace ADAMTS13, the mainstay of treatment for acquired TTP is plasma exchange, which is additionally thought to remove the inhibitory autoantibodies. Although this therapy carries considerable morbidity including exposure to blood products from multiple donors, plasma exchange has reduced the mortality of acquired TTP from 90% to approximately 20%. Unfortunately, about one-third of acquired TTP cases become chronic and significant morbidity remains a clinical challenge.

Children with familial TTP often present in the perinatal period with severe unconjugated hyperbilirubinemia and a Coombs negative hemolytic anemia. The peripheral blood smear reveals thrombocytopenia and signs of microangiopathic hemolytic anemia similar to patients with acquired TTP. As above, in contrast to patients with acquired TTP, patients with familial TTP often respond well to simple plasma infusion, but nearly all will relapse.

TTP-like conditions also occur in association with a variety of clinical conditions such as cancer, bone marrow transplantation, collagen vascular disease, and specific antiplatelet drugs. Interestingly, many of these conditions seem to occur in the absence of ADAMTS13 deficiency, suggesting perhaps differing mechanisms of pathogenesis. Thrombotic microangiopathy in pregnancy deserves special mention as pregnancy has long been recognized as a prothrombotic state that seems to be a particular risk factor for triggering episodes of both familial TTP and acquired TTP.

Mutations in ADAMTS13 Cause Human Familial TTP

To date more than 50 mutations in ADAMTS13 have been identified in patients with familial TTP. Approximately 60% of these are missense single amino acid substitutions, with the remaining 40% being nonsense, frameshift, or splice site mutants expected to result in a truncated protein. Although the mutations that result in a truncated protein are equally distributed throughout the molecule, more than 75% of the missense mutations cluster in the first half of ADAMTS13. In vitro studies of several missense mutants demonstrate impaired secretion into cell culture media. These studies indicated that impaired secretion into the circulation may be the dominant mechanism of ADAMTS13 dysfunction and suggested that most patients with familial TTP will have absent or nearly absent ADAMTS13 antigen levels, which with the development of new ADAMTS13 antigen ELISA assays indeed appears to be the case.

To date, no clear genotype-phenotype correlation has been established for patients with familial TTP, and there is no evidence for locus heterogeneity (ie, there does not appear to be a second gene). Interestingly, ADAMTS13 mutations have been found in patients diagnosed initially with atypical hemolytic uremic syndrome (HUS), idiopathic thrombocytopenic purpura, and Evans syndrome, suggesting that in many cases the diagnosis of familial TTP is missed, and the true incidence of this disease may be underestimated.

Is ADAMTS13 Deficiency Sufficient to Cause TTP?

Analysis of a large cohort of patients with familial TTP revealed an age-dependant clustering of cases into 2 relatively distinct groups. About 50% of patients presented within the first 5 years of life, but a second group remained without symptoms until 20 to 40 years of age. Examples of siblings with the same ADAMTS13 mutations but markedly different clinical courses have also been reported. Thus, in the case of familial TTP, ADAMTS13 deficiency in and of itself does not seem to be sufficient for disease pathogenesis.

ADAMTS13 deficiency also does not appear to be sufficient for pathogenesis of acquired TTP. For example, many patients with acquired TTP who achieve clinical remission with plasma exchange continue to show a persistent severe deficiency in ADAMTS13 activity or the persistence of a significant ADAMTS13 plasma inhibitor. Similar to patients with familial ADAMTS13 mutations who have not yet developed clinically apparent TTP, these patients with acquired TTP in remission are without symptoms despite ongoing severe ADAMTS13 deficiency.

These observations demonstrate that in addition to ADAMTS13 deficiency, additional genetic or environmental factors are required for the pathogenesis of both familial and acquired TTP. Environmental triggers may include infections, pregnancy, surgery, bone marrow transplant, and certain medications; whereas possible genetic factors may include those associated with regulation of the coagulation cascade, vWF, or platelet function, or components of the endothelial vessel surface.

An excellent example of this principle was uncovered recently through analysis of 2 siblings with the same ADAMTS13 mutations but dramatically different clinical TTP courses. Besides harboring a mutation in ADAMTS13, the sibling with the more severe clinical course, including extensive renal disease and eventually death attributable to cerebral stroke, was found also to carry a heterozygous mutation in the gene encoding complement factor H. Interestingly, mutations in this gene and others encoding additional complement regulatory proteins are known to cause atypical HUS, a thrombotic microangiopathy closely related to TTP. Therefore in this case, it appeared that the mutations in ADAMTS13 and factor H combined to result in a more severe phenotype than expected from either alone. This observation suggests that factors that control complement regulation may serve as modifiers of TTP susceptibility, and additionally that TTP and HUS may share some degree of an overlapping mechanism of pathogenesis.

Regarding pathogenesis of acquired TTP, it remains unclear what triggers the formation of the inhibitory autoantibodies against ADAMTS13. Although the autoantibody response is polyclonal in nature, the critical epitopes required...
for ADAMTS13 inhibition appear to reside within the spacer and cysteine-rich domains of ADAMTS13. Fine epitope mapping and functional characterization of the different inhibitory autoantibodies is a subject of considerable research interest. Additional questions remain as to the nature of the environmental factors involved in the relapse acquired TTP and whether clinical parameters might help identify those patients at risk for relapse.

Establishing a Mouse Model of TTP
For many years lack of a suitable animal model has greatly hampered researchers investigating the pathophysiology of TTP. However, the recent identification of ADAMTS13 mutations in patients with familial TTP opened the door for the development of a "knock-out" mouse model of ADAMTS13 deficiency, and 2 groups (including ours) generated ADAMTS13-deficient mice by gene targeting. Our group initially studied these mice on a mixed genetic background comprising the common laboratory mouse strains C57BL/6 and 129x1/Sv. Our initial intercross of mice heterozygous for the ADAMTS13 knock-out allele (Adamts13/H11002) generated offspring in the expected Mendelian ratio (25% Adamst13/H11002, 50% Adamst13/H11002, and 25% Adamts13/H11002) indicating no loss of ADAMTS13-deficient (Adamts13/H11002) mice in utero, or in the immediate postnatal period. Examination of plasma ADAMTS13 function confirmed complete loss of vWF-cleaving activity in the Adamts13/H11002 mice, with normal activity observed in the Adamts13/H11002 (50% activity in the heterozygotes).

We subsequently undertook a thorough examination of these original Adamts13/H11002 mice for findings consistent with TTP, which surprisingly (at least at the time) were not present. Peripheral blood counts and blood smears were normal without signs of hemolytic anemia or thrombocytopenia, and there was no evidence of hyaline thrombus formation on histological examination of numerous TTP target organs. Finally, 1-year Kaplan-Meier survival rates were not statistically different from wild-type or heterozygous littermate controls. Curiously, when we examined the vWF multimer profile in these mice, there was no difference between wild-type and ADAMTS13-deficient animals. Furthermore, vWF multimers from both genotypes appeared similar in size to UL-vWF from familial TTP patient plasma. Thus mice of this genetic background (mixed C57BL/6 and 129x1/Sv) exhibited UL-vWF regardless of whether or not they expressed ADAMTS13.

As mentioned previously, a second group of investigators also developed ADAMTS13-deficient mice, in this case on a pure strain 129x1/Sv genetic background. Similar to our ADAMTS13-deficient mice on the mixed genetic background, these independently-derived Adamts13/H11002 mice also failed to demonstrate any findings consistent with TTP. However, when the vWF multimer profile was examined in these mice, there was a clear difference with the Adamts13/H11002 mice exhibiting UL-vWF, and the Adamts13/H11002 controls demonstrating a normal multimer pattern. Thus on the pure strain 129x1/Sv genetic background, ADAMTS13 deficiency resulted in the appearance of UL-vWF, but without any evidence of TTP.

Despite lack of TTP findings in either ADAMTS13-deficient mouse line, with further careful examination, both groups were able to demonstrate slight phenotypic differences between ADAMTS13-deficient and wild-type mice. Using intravital microscopy and infusion of fluorescent platelets, we demonstrated that vWF-mediated interactions between platelets and the vascular endothelium were significantly prolonged in ADAMTS13-deficient mice compared with wild-type controls. Similarly, when the pure-strain 129x1/Sv ADAMTS13-deficient mice were challenged with the platelet and endothelial agonists collagen and epinephrine, the ADAMTS13-deficient mice developed a more severe thrombocytopenia than their wild-type control littermates, although mortality between the 2 groups was not different.

Thus at this point it seemed that ADAMTS13 deficiency in mice resulted in subtle differences in vWF-mediated platelet function, but was not sufficient to bring about the full pathogenesis of TTP. Did this lack of TTP findings indicate that the ADAMTS13-deficient mice were a poor model for the human disease? Or more optimistically, were the ADAMTS13-deficient mice recapitulating the situation in humans where deficiency of ADAMTS13 is not sufficient for TTP pathogenesis? In this case, perhaps, the asymptomatic ADAMTS13-deficient mice might provide an ideal system for investigating potential genetic modifying factors and environmental triggers for this important disease.

Influence of Genetic Background on TTP Pathogenesis in the Mouse
It is becoming increasingly clear that the phenotypes of "knock-out" and transgenic mice can vary extensively among different genetic backgrounds, even to the point where a homozygous knock-out allele may be embryonic lethal in one strain and show no phenotype in another. With this in mind, we crossed our Adamts13/H11002 mice with mice of the CASA/Rk genetic background. We chose the CASA/Rk strain because of its large genetic difference from C57BL/6 and 129x1/Sv, and the previous observation that CASA/Rk mice exhibit plasma vWF levels significantly higher than C57BL/6 and 129x1/Sv mice. We considered this latter point especially important as the high degree of vWF level variation in both humans and mice, coupled with its function as the only known substrate of ADAMTS13, suggested genetic factors that regulate vWF level as possible modifiers of TTP susceptibility.

These new ADAMTS13-deficient mice were generated by crossing our original mixed-background Adamts13/H11002 mice with wild-type CASA/Rk mice (for 2 generations), followed by a subsequent intercross to obtain Adamts13/CASA/H11002, Adamts13/CASA/H11002, and Adamts13/CASA/H11002 offspring. Again, these mice were born with the expected Mendelian ratio indicating no loss of ADAMTS13-deficient mice in utero or in the immediate postnatal period. However, this is where the similarity with the original ADAMTS13-deficient mice ended. First, complete blood count analyses demonstrated that 20% of the Adamts13/CASA/H11002 mice were severely thrombocytopenic, compared with 0% of littermate controls. Second, in contrast to our Adamts13/H11002 mice de-
scribed above, the *Adams13/CASA*+/+ mice did not exhibit UL-vWF, and indeed demonstrated a multimer distribution similar to normal humans. In addition, ADAMTS13 deficiency now resulted in the appearance of UL-vWF in the *Adams13/CASA*−/− mice.

It next became clear that a subset of the *Adams13/CASA*−/− mice went on to become sick-appearing and exhibit frank clinical signs of TTP including thrombocytopenia and microangiopathic hemolytic anemia on peripheral blood smear (Figure 1A), and vWF-rich/fibrin-poor thrombi in multiple organs including the brain, heart (Figure 2), and kidneys (not shown). Finally, as cohorts of these mice were followed over time, the *Adams13/CASA*−/− mice demonstrated a significantly increased mortality rate, with a 6-month Kaplan-Meier survival probability of <60% (compared with >90% survival for their wild-type and heterozygous littermates).

Thus interestingly, introduction of the CASA/Rk genetic background appeared to render susceptibility to TTP in the setting of ADAMTS13 deficiency. Additionally, in many respects the *Adams13/CASA*−/− mice resembled humans with familial ADAMTS13 deficiency, having a variable time to presentation, and with thrombocytopenia typically preceding development of clinically overt TTP.

**Examining Potential Environmental Triggers of TTP in the Mouse**

Another advantage to using mouse models to study the pathophysiology of human disease is the ability to manipulate the environment to investigate potential disease triggers. In the case of TTP, endothelial injury has long been postulated to play a role in disease pathogenesis. Furthermore, the clinical presentation of TTP can be very similar to a closely-related thrombotic microangiopathy referred to as the hemolytic-uremic syndrome (HUS). Most cases of acquired HUS are caused by infection with strains of bacteria (typically *E. coli* O1:H157 in North America) that elaborate shigatoxins (Stx), substances known to be toxic to endothelial cells. Therefore we investigated the effect of Stx in the ADAMTS13-deficient mice on the TTP-sensitive genetic background and their wild-type littermate controls (*Adams13/CASA*−/− and *Adams13/CASA*+/+). Interestingly, Stx induced findings consistent with TTP in the *Adams13/CASA*−/− mice similar to those described above (but not in the controls), including variable degrees of thrombocytopenia, microangiopathic hemolytic anemia (Figure 1B), and vWF-rich/fibrin-poor hyaline thrombi in the brain, heart, and kidneys (nearly identical to those shown in Figure 2). Furthermore, these findings were not observed with Stx challenge of our original ADAMTS13-deficient mice on the mixed C57BL/6 and 129x1/Sv background, demonstrating again the importance of genetic modifying factors in this model animal system.

**Does vWF Level Affect TTP Pathogenesis in the Mouse?**

To address the hypothesis that an increase in plasma vWF level was responsible for the heightened susceptibility of the *Adams13/CASA*−/− mice to TTP pathogenesis, we first measured plasma vWF level in these mice, which was found to be increased 50% on average over that of the original ADAMTS13-deficient mice on the mixed C57BL/6 and 129x1/Sv background. However, because the *Adams13/CASA*−/− mice had undergone only 2 generations of matings with wild-type CASA/Rk mice, they were expected to exhibit a wide range of vWF levels, as any individual mouse would...
have inherited a random combination of vWF-regulating factors from either the original C57BL/6 and 129x1/Sv background, or from the new CASA/Rk background. Indeed this was the case as vWF values of individual \textit{Adamts13/CASA}^{−/−} mice ranged from 200% to 600% compared with wild-type C57BL/6 mice. We next hypothesized that this variability could be used to investigate whether increased plasma vWF level affects TTP pathogenesis, as the mice that inherited the highest vWF levels would be expected to demonstrate the greatest response to Stx, whereas those that inherited the lowest levels would be expected to respond poorly to Stx or not at all. Surprisingly, however, we found no correlation between plasma vWF level and degree of Stx-induced thrombocytopenia or mortality. These findings demonstrated that elevated plasma vWF (possibly above a required threshold level) is not a risk factor for TTP in this model system, and that other genetic modifying factors gained or lost as a result of the CASA/Rk cross likely are responsible for the TTP susceptibility of the \textit{Adamts13/CASA}^{−/−} mice.

**A Mouse Model of Acquired TTP?**

Our discussion thus far has focused on mouse models of familial \textit{Adamts13} deficiency. Investigators have also used mice to investigate the function of anti-\textit{Adamts13} antibodies in a mouse model.\textsuperscript{46} Using intravital microscopy, \textit{Adamts13}^{+/−} mice (on the non-TTP susceptible genetic background) receiving an injection of polyclonal rabbit anti-human \textit{Adamts13} antisera demonstrated significantly prolonged vWF-mediated platelet-endothelial interactions, similar to control \textit{Adamts13}^{−/−} mice not receiving antisera. Thus the anti-\textit{Adamts13} antisera was able to induce functional \textit{Adamts13} deficiency, possibly in a manner similar to humans with acquired TTP. Although the antibodies used in these experiments were not isolated from a patient with acquired TTP, these experiments demonstrated that antibody-mediated inhibition of \textit{Adamts13} in the mouse could possibly be used to model this form of TTP.

**Discussion**

The existence of a mouse model for \textit{Adamts13} deficiency opens up many new doors for investigations regarding the pathophysiology of TTP. The \textit{Adamts13/CASA}^{−/−} mice represent the only small animal model of which we are aware that faithfully recapitulates the key clinical findings of human TTP. Therefore, it may be possible to use these mice to screen for pharmacological agents that affect the development of both spontaneous and induced TTP. The \textit{Adamts13}-deficient mice also provide a natural tool for testing the efficacy of recombinant \textit{Adamts13} products, which in addition to the wild-type form, could conceivably be engineered to lack epitopes that are the targets of the inhibitory auto-antibodies seen in acquired TTP, for the possible treatment of this disorder.\textsuperscript{47}

The identification of environmental triggers for TTP is a subject of considerable research interest as it may provide new insights into both pathogenesis and treatment. In our studies we identified that administration of the bacterial agent Stx precipitates TTP in the setting of murine \textit{Adamts13} deficiency on a susceptible genetic background. As Stx is a well-documented environmental trigger for the closely-related thrombotic microangiopathy HUS, this observation brings up several interesting questions and possibilities. First and importantly, the fact that Stx is able to trigger TTP under certain circumstances suggests a shared mechanism of pathophysiology between these 2 disorders. One might ask why Stx did not trigger HUS in our model. Although this question is being pursued in our laboratory, currently we speculate that in the case of systemic \textit{Adamts13} deficiency, the predominant clinical picture is TTP resulting from the widespread formation of vWF and platelet thrombi throughout the arterial circulation of multiple organs. On the other hand, in the case of systemic \textit{Adamts13} sufficiency, the Stx-induced pathology may be principally restricted to the kidneys, perhaps relating hypothetically to a decreased ability of \textit{Adamts13} to cleave vWF released in the glomerular microcapillary circulation. If the former speculation were correct, we might expect that infection of a person with familial TTP with shiga-toxigenic \textit{E coli} (STEC) would trigger a TTP flare. However, to our knowledge, this occurrence has not been reported.

Regarding mouse models of HUS, attempts to trigger this disease with Stx alone in common strains of laboratory mice have not been successful, suggesting the possible requirement for a susceptible genetic background. Another factor regarding Stx-induced TTP versus HUS in the mouse may be the route of Stx administration. In humans with HUS triggered by STEC infection, Stx levels remain undetectable in the circulation. Neutrophils are thought to bind Stx locally at the site of infection in the GI tract, and subsequently transport it predominantly to the renal endothelium with Stx receptors are perhaps the most concentrated.\textsuperscript{48–50} Is important to note that Stx may be delivered to lesser degrees elsewhere, as thrombi are often seen in HUS in locations apart from the kidneys. In our experiments, we provided Stx to the \textit{Adamts13}-deficient mice intravenously, therefore allowing it access to all endothelial beds, possibly resulting in the predominant clinical TTP picture. These questions are being addressed in our laboratory.

Our use of Stx was not intended to further blur the already difficult clinical distinction between TTP and HUS, but rather to expose the \textit{Adamts13}-deficient mice to an agent known to be toxic to endothelial cells. Endothelial injury has long been postulated to be a trigger for TTP.\textsuperscript{1} The toxic effects of Stx on endothelial cells have been well documented,\textsuperscript{48,49,51} and moreover recently it was demonstrated that Stx can also stimulate the release of vWF from cultured endothelial cells at doses that do not to induce rapid cell death.\textsuperscript{52} This latter observation has several important implications for both HUS and TTP. First, regarding HUS, it is suggestive that Stx-induced vWF release by the renal glomerular endothelium may contribute to the pathogenesis of STEC-induced HUS. Additionally, when coupled with our observation that Stx induces TTP in \textit{Adamts13}-deficient mice, the fact that Stx can apparently also function as an endothelial agonist suggests that other substances similarly able to affect endothelial cell activation may be potential triggers for TTP (and possibly HUS). Such compounds might include known endothelial...
agonists such as ddAVP, histamine, epinephrine, thrombin, and certain cytokines. These questions are also being addressed in our laboratory.

In addition to facilitating the identification of additional environmental triggers for TTP, the ADAMTS13-deficient mice may also prove helpful in identifying potential genetic modifying factors for this important disorder. In this regard we are currently pursuing identification of the genetic modifying factor(s) responsible for the change from the TTP-resistant (the original mixed background C57BL/6 and 129x1/Sv mice), to the TTP-sensitive (the Adamts13/CASA mice) phenotype. Theoretically, this change could have resulted from either the gain of a susceptibility gene (or genes) from CASA/Rk, or the loss of a protective gene (or genes) from either C57BL/6 or 129x1/Sv. Whichever the case, we are performing additional crosses followed by whole genome scanning and linkage analyses to identify the genetic variations that account for the differences in TTP-susceptibility. Similar experiments attempting to identify disease modifier genes in humans are inherently difficult to perform. Another advantage of using this approach to identify genetic modifying factors is its inherent lack of bias, which may identify the first function of a novel gene, or a new role for a gene with a previously-established function.

Before the genetic linkage and positional cloning studies are completed, we can only speculate on possible reasons underlying the strain-specific TTP susceptibility identified in our studies. As mentioned above, we hypothesized initially that the TTP-susceptibility introduced by CASA/Rk was attributable the elevated plasma vWF levels characteristic of this strain. However, experiments correlating degree of both spontaneous and Stx-induced TTP pathology with plasma vWF level did not support this hypothesis. Therefore at this point we speculate that the putative CASA/Rk determinants of TTP susceptibility may reside in other genetic factors, perhaps associated with endothelial or platelet function.

Another advantage of murine models of human disease is the ability to examine roles that other potentially important molecules play in pathogenesis of the disease under investigation. For example, despite the critical role postulated for vWF in TTP pathogenesis, direct experimental evidence in support of this hypothesis is lacking. We now are in a position to formally address this question by crossing the Adamts13-deficient mice with vWF-deficient mice to generate ADAMTS13/vWF “double knockouts”. After these mice are generated, they will be further crossed onto the TTP-susceptible CASA/Rk background, and the resulting mice will be assessed for both spontaneous and Stx-induced TTP. If findings consistent with TTP are no longer observed, this would provide direct evidence that vWF is indeed required for TTP pathogenesis. This type of formal proof would be difficult to demonstrate in a nongenetic system.

Other candidate mouse lines including those with complement factor H deficiency (implicated in pathogenesis of atypical HUS in humans) and those harboring the coagulation Factor V Leiden mutation (a well-known human thrombotic risk factor) could also be used to investigate the potential contribution of these genes to TTP susceptibility and pathogenesis.

As a final point, we will discuss the interesting differences in the vWF multimer patterns noted among the various mouse lines described above. Recall that Adamts13+/− mice of the mixed C57BL/6 and 129x1/Sv genetic background exhibited UL-vWF, whereas the pure strain 129x1/Sv Adamts13+/− mice and the Adamts13/CASA+/− mice did not. It is important to note C57BL/6 mice primarily express a form of ADAMTS13 that is truncated after the sixth TSP-1 domain, which resulted from insertion of a retrovirus-like element into the Adamts13 gene.53 129x1/Sv and CASA/Rk mice both express full-length ADAMTS13. However, the C57BL/6 and 129x1/Sv mixed background Adamts13+/− mice all express truncated ADAMTS13 derived originally from their C57BL/6 background (because the knock-out Adamts13 allele of the founder mice of this line was obligatorily derived from the 129x1/Sv component).

This truncated form of ADAMTS13, termed ADAMTS13S, lacks the distal one-fourth of this molecule, including the seventh and eighth TSP-1 motifs, and the 2 CUB domains. Although ADAMTS13S retains grossly normal activity in vitro when tested against an artificial 73-aa vWF substrate, its activity against full-length multimeric vWF recently has been determined to be considerably reduced.54 Additionally, studies in vitro suggest that ADAMTS13 binding to multimeric vWF may be mediated in part through the distal TSP-1 and CUB domain of ADAMTS13.54,55 Thus we speculate that the truncated form of ADAMTS13 may be hypomorphic in vivo, potentially accounting for the presence of UL-vWF observed in the mixed background Adamts13+/− mice (which express ADAMTS13S).

Since first described by Eli Moschcowitz in 1924, TTP has fascinated clinicians and researchers alike. Much of this interest with TTP likely relates to its often sudden and dramatic appearance in previously-healthy individuals, its rapidly progressive and often fatal course, and the fact that timely diagnosis and rapid treatment can truly be life saving. The last several decades have brought many exciting discoveries to the field, culminating in the molecular cloning of ADAMTS13 and identification of its role in TTP pathogenesis. More recently, the understanding that ADAMTS13 deficiency is necessary, but not sufficient, for the development of most cases of TTP has opened the door to the identification of environmental triggers and genetic modifying factors that surely play important additional roles in disease pathogenesis. Finally, the development of a mouse model for this disorder has the potential to contribute to nearly all areas of TTP research, including the direct study of disease pathophysiology, and the ability to serve as an important tool for the screening of potential therapeutics and recombinant ADAMTS13 products, and the further identification of potential environmental triggers and genetic modifying factors. This is an exciting time in the field of TTP research, and we are optimistic that the next several years will contribute greatly to both our understanding and treatment of this intriguing hematologic disease.

Disclosures

None.
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