

**Blood’s 70th anniversary: the elusive von Willebrand factor–cleaving protease**

As part of a year-long celebration in honor of Blood’s 70th anniversary, we are publishing a series of editorials written by past Editors-in-Chief of the journal. The authors reflect on their experience at Blood in light of the journal’s publication history. Each of these special pieces will highlight and discuss the impact of one or more original research articles that had a significant influence on the field or that mark a pioneering scientific development in hematology that appeared in the journal during the author’s term as Editor-in-Chief.

In 1924, Eli Moschowitz described a 16-year-old girl who developed pallor, petechial bleeding and hematuria, fever, and progressive paralysis and coma, dying within 2 weeks.1 At autopsy, many organs were compromised by arteriolar and capillary thrombi, composed primarily of platelets. This disease was soon recognized as Moschowitz disease, but in 1947 was renamed thrombotic thrombocytopenic purpura (TTP) based on the growing recognition of the pathological appearance of the affected organs. For the 40 to 50 years following its description, the origin of TTP was completely unknown and was nearly uniformly fatal.

In 1966, Amorosi and Ullmann penned a review of 271 patients with TTP,2 defining the classic pentad of clinical findings in the disorder: microangiopathic hemolytic anemia, thrombocytopenia, fluctuating altered mental status, fever, and variable degrees of renal failure. Over the ensuing decades, clinicians came to realize that the “classic pentad” was only very rarely seen; rather, given the near fatal outcome of the disorder left untreated, and the burgeoning of effective therapy for TTP, clinicians realized that 4 or even 3 of the classic pentad of symptoms and signs should suffice for the clinical diagnosis of TTP, and treatment begun empirically. It was also realized that many patients underwent a relapsing/remitting course, with relapse often heralded by recurrence of isolated thrombocytopenia. However, very little remained known of the pathophysiology of the disease, and a reliable diagnostic test was lacking.

Following on the description by Erik von Willebrand of a severe congenital bleeding disorder in the Åland Islands in 1926,3 von Willebrand factor (VWF) was identified as a critical procoagulant molecule involved in platelet adhesion to the injured vascular wall, and was ultimately purified and cloned in 1985 by Evan Sadler et al.4 In 1982, Joel Moake and colleagues reported the presence of ultra-high-molecular-weight forms of VWF (UHVVWF) in patients experiencing an acute episode of TTP,3 which reverted to the size distribution found in normal plasma when patients were successfully treated and entered remission. Because VWF had been shown to be a highly polymeric protein, and the ultra-high-molecular-weight forms in the plasma of patients with TTP were similar in size to the VWF that is produced in endothelial cells, these investigators went on to postulate that the lack of a “depolymerase” was responsible for the presence of the UHVVWF, which was, in turn responsible for disseminated thrombosis found in patients with TTP. The search for the “depolymerase” was on!

A number of subsequent findings suggested that the responsible enzyme was a protease not a “depolymerase.” In 1998, independent reports by Miha Furlan et al at University Hospital of Bern (Bern, Switzerland)5 and by Tsai and Lian at Albert Einstein School of Medicine (Bronx, NY)6 appeared back to back in the New England Journal of Medicine describing an immunoglobulin fraction of plasma from patients with acute TTP, not present in remission samples, that inhibited the ability of barium (which is known to activate many proteases) to degrade plasma VWF to low-molecular-weight forms. Apparently, autoantibodies either inactivated or cleared the “VWF-cleaving protease.”

In 2001, a manuscript describing the purification and primary amino acid sequence of a VWF-cleaving protease was submitted to Blood by one of the same groups (Furlan et al) that had 3 years earlier identified the inhibitory antibodies to the protease. As Editor-in-Chief of Blood, understanding that the literature is rife with single publications claiming the identification of a highly sought-after molecule and knowing a large number of investigators attempting to purify the VWF-cleaving protease, I queried more than half a dozen different laboratories to gauge their success in identifying the protease. One laboratory, 3 flights of stairs from my own, was the only one to claim success: Kazuo Fujikawa, in the laboratory of Earl Davie at the University of Washington, told me rather nonchalantly, “Yes, we have the sequence.” After thorough peer reviews, we published both papers, back to back (in the order of receipt) in September 2001.8,9 A month later, based on a successful positional cloning effort in 4 families with congenital TTP (Upshaw-Schulman syndrome), Gallia Levy, in the laboratory of David Ginsburg at the University of Michigan, reported in the journal Nature10 on the causative genetic abnormality; the full-length complementary DNA predicted a protease of the ADAMTS (a disintegrin and metalloprotease with thrombospondin repeats) family that precisely matched the purified protein sequences of Furlan and Fujikawa published in Blood the month before. In Fall 2001, there remained little or no doubt that the VWF-cleaving protease was ADAMTS13, and that its absence or inhibition is responsible for TTP, at least in large part.

Since those heady days of 2001, over 600 publications on the biology and clinical utility of ADAMTS13 have been published. We now know a great deal about ADAMTS13: it is composed of an amino-terminal catalytic domain, a disintegrin domain, a thrombospondin type-1 (TSP-1) repeat, a cysteine-rich spacer domain, a carboxyl-terminal region with 7 additional TSP-1 repeats, 2 unique CUB (complement C1r/C1s, Uegf [EGF-related urchin protein], BMP-1 [bone morphogenic protein]) domains, and several metal-binding domains necessary for catalytic activity.11 The protease is produced by hepatic stellate cells, endothelial cells (which cleave the secreted, tethered surface UHVWF), and megakaryocytes.12 Our understanding of the normal processing of the UHVWF secreted by endothelial cells and megakaryocytes has been rapid. We now know...
that upon secretion the UHVWF is tethered to the cell surface, making it susceptible to limited ADAMTS13 proteolysis in the presence of coagulation factor VIII and platelets, yielding the 500 kDa–to 20 MDa–sized forms that normally circulate in the plasma. ADAMTS13 binds to VWF and cleaves in the A2 domain of the substrate, although this binding site appears to be cryptic, unveiled by shear stress applied to the VWF molecule. We now also appreciate that mutations of VWF that make it more susceptible to ADAMTS13-induced proteolysis result in one of the forms of type 2 von Willebrand disease. Additional binding sites for the protease under active investigation.

Clinically, although a disease resembling TTP can be reproduced by genetic elimination of ADAMTS13 in mice, the murine disease is strain specific, and can be triggered by bacterial toxins similar to that responsible for the (clinically overlapping) hemolytic uremic syndrome, indicating that ADAMTS13 is necessary but not sufficient for TTP, at least in mice. The availability of an ADAMTS13 immunoassay has allowed a more precise diagnostic approach to the patient with thrombocytopenia, and recombinant ADAMTS13 is undergoing clinical evaluation as a therapeutic, not only for the solution to perplexing clinical problems of humans. And these works serve to point out that regulation of the size and function of VWF can result in bleeding (type 2 VWD) or thrombosis (TTP), pointing out, yet again, the delicate balance of the hemostatic system. And these works serve to point out the great excitement that can be found in modern medicine: the hunt for the solution to perplexing clinical problems of humans.

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**References**


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