

# Current understanding of the pathophysiology of thrombotic thrombocytopenic purpura

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Thrombotic thrombocytopenic purpura (TTP) was first described by Moschowitz in 1924.<sup>1</sup> Although it is now well recognised, being characterised by the classic pentad of fever, thrombocytopenia, microangiopathic haemolytic anaemia (MAHA), renal impairment, and fluctuating neurological signs, its precise pathophysiology remains elusive. Several other syndromes including haemolytic uraemic syndrome (HUS), eclampsia and HELLP (haemolysis, elevated liver enzymes, and low platelets) are also characterised by MAHA and this has given rise to the concept that they might represent a spectrum of disease. However, recent independent work by Tsai and Furlan indicates that TTP and HUS appear to be distinct pathophysiological entities, which might explain in part the wide variation in response to treatment that is seen in clinical practice.

## Clinical variants

Several variants of TTP are recognised. Most cases are defined as single episode TTP: in these patients there is no identifiable precipitant and no subsequent recurrence. Because survival has improved with advances in treatment, it is now apparent that a few patients continue to relapse at infrequent intervals: in one study it was estimated that 11–36% fall into this category, known as intermittent TTP (fig 1), with relapses occurring up to eight years after the index episode.<sup>2</sup> In some instances, the index or subsequent episode might be precipitated by an identifiable cause, resulting in secondary TTP. Several drugs have been implicated including mitomycin C, ticlopidine, cyclosporin, oral contraceptives, and quinine. Perhaps those most closely linked are ticlopidine and cyclosporin. Ticlopidine characteristically results in TTP after two to four weeks of use,<sup>3</sup> whereas cyclosporin is a recognised risk factor in post-allogeneic bone marrow transplant associated TTP, along with total body irradiation conditioning. Pregnancy and systemic lupus erythematosus (SLE) are other associations and might account, in part, for the female preponderance (male to female ratio, 1 : 2). Anecdotally, TTP also appears to be triggered by viral infections. However, because fever is one constituent of the defining pentad of TTP, such symptoms might actually represent a prodromal TTP illness. Nevertheless, certain infections are associated: HIV infection, especially when associated with a high viral load, is one example. Of interest, a TTP like illness in association with *Bartonella bacilliformis* like organisms adherent to 0.1–2% of circulating erythrocytes has been reported in five patients.<sup>4</sup> It would appear, however, that

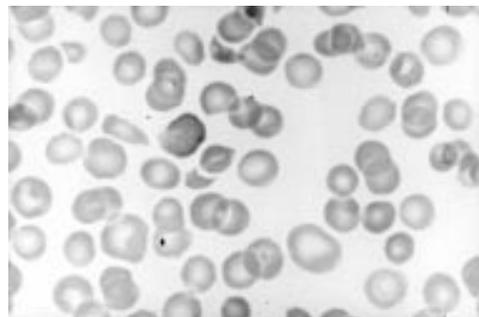


Figure 1 Peripheral blood film of patient with intermittent thrombotic thrombocytopenic purpura (TTP). Severe thrombocytopenia and red cell fragmentation are prominent features. Circulating normoblasts and neutrophilia may also be seen.

infectious precipitants are relatively uncommon because TTP is not associated with seasonal variation and case clustering rarely occurs. Further research into this area is awaited.

Rarely, despite treatment, there is evidence of continued haemolysis, as shown by persistently raised lactate dehydrogenase (LDH) in combination with a reticulocytosis and thrombocytopenia. This is classified as chronic unremitting TTP, which should be differentiated from chronic relapsing TTP. The latter is a congenital condition usually presenting in infancy or early childhood and is characterised by frequent episodes of haemolysis at predictable intervals (usually every 21–30 days). In contrast to single episode or intermittent TTP, these episodes normally respond to infusion of fresh frozen plasma and there is no requirement for concurrent plasmapheresis.<sup>5</sup>

## Histology

Regardless of the exact nature of TTP, be it congenital or acquired, the histological findings appear to be identical. Microvascular thrombi are the predominant abnormality, and are found primarily in the renal and cerebral circulation, thus accounting for the clinical features of the disease (fig 2). However, other anatomical sites can be affected—for example, abdominal pain and serous retinal detachment are recognised complications of TTP and are thought to be secondary to mesenteric and retinal ischaemia, respectively.

The microvascular thrombi formed have been shown to be composed almost entirely of platelets. Because neither pronounced perivascular inflammation, obvious endothelial cell desquamation, nor subendothelial exposure have been demonstrated in TTP, it has been hypothesised that TTP might be a disease of primary platelet aggregation within the

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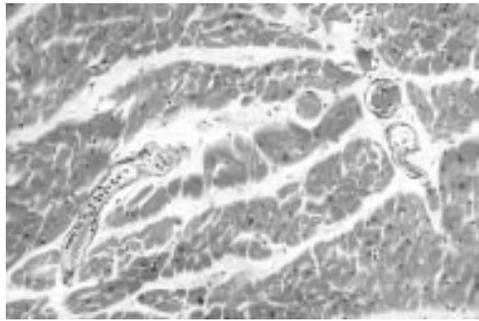


Figure 2 Typical histological findings of acute thrombotic thrombocytopenic purpura (TTP) (cardiac muscle). There is florid thrombosis within the microvasculature. Immunohistochemistry shows these thrombi to be composed predominantly of von Willebrand factor (vWF) and platelets, with only small amounts of fibrin and fibrinogen.

microvasculature.<sup>6</sup> In support of this, immunohistochemistry shows that thrombi formed in TTP contain abundant amounts of von Willebrand factor (vWF) and little fibrinogen or fibrin, in contrast to those found in disseminated intravascular coagulation (DIC), where the reverse is seen.<sup>7</sup> This has led to the hypothesis that vWF multimers, perhaps in conjunction with high shear stress, might promote platelet aggregation during episodes of TTP.

#### Platelet aggregation

Platelets form aggregates through sequential stages of adhesion, activation, and aggregation. Although platelets circulate in close proximity to the endothelium, intravascular aggregation normally only occurs after endothelial damage. Subendothelial vWF is then exposed and binds to the platelet glycoprotein (Gp) Ib-IX-V receptor. This interaction has an extremely fast association rate and therefore proceeds despite the high shear pressures that exist within the microvasculature. Platelet activation results and, as a consequence, the platelet GpIIb/IIIa receptor undergoes a conformational change, which allows binding not only to surface bound fibrinogen, but also to free fibrinogen. Crosslinkage of platelets follows, secondary to the dimeric nature of fibrinogen, and aggregation results. However, if platelets are exposed to high shear pressures, direct platelet aggregation may occur in the fluid phase. This phenomenon has an absolute requirement for vWF, GpIb, GpIIb/IIIa, calcium, and ADP.<sup>8-10</sup>

Evidence for the importance of direct platelet aggregation in the pathogenesis of TTP has been provided experimentally: a cone and plate viscometer minimises platelet surface interactions and allows direct platelet aggregation under raised shear fluid stresses. Using this model, it has been demonstrated that platelet rich plasma from patients with congenital TTP results in excessive platelet aggregation, as compared with normal adult platelet rich plasma samples, when exposed to shear stresses of 90–180 dyne/cm<sup>2</sup> (similar to those found in partially occluded microvasculature).<sup>11</sup> Mixing studies suggest that this is caused by a plasma component rather than being secondary to a platelet defect. More specifically, ultra large forms of vWF (ULvWF) are implicated because, not only are they



Figure 3 von Willebrand factor (vWF) multimeric analysis demonstrating unusually large forms of vWF (ULvWF) in patient plasma obtained during an episode of thrombotic thrombocytopenic purpura (TTP) (lane 1, marked with an arrow). By comparison, lane 2 shows the series of vWF multimeric bands present in normal pooled platelet poor plasma: ULvWF are not a feature.

present in TTP plasma (fig 3), but the addition of ULvWF to normal plasma accentuates platelet aggregation. Similarly, platelet retention, as measured by the modified Adeplat S test (a glass bead retention method) at the relatively modest shear stress of 76 dyne/cm<sup>2</sup>, is increased in congenital TTP compared with normal controls. In this case, loss of ULvWF is associated with normalisation of increased platelet retention, again supporting the concept that vWF multimers might play an integral role in TTP.<sup>12</sup>

#### Von Willebrand factor

vWF circulates in the plasma as a series of multimers of 500–20 000 kDa and is composed of 270 kDa monomers linked by disulphide bonds. Although it is synthesised in megakaryocytes and stored within platelet  $\alpha$  granules, the predominant source of plasma vWF is the endothelium, where it is stored within the Weibel-Palade bodies. ULvWF is found in endothelial cells and platelets and probably consists of an increased number of mature vWF subunits because pro-vWF monomers do not appear to be a constituent.<sup>13</sup> ULvWF multimers are secreted in a retrograde direction from endothelial cells to the vascular subendothelium, where they are protected from proteolytic cleavage. If ULvWF do enter the circulation they have been shown to be superior to the largest plasma vWF forms at binding under the influence of fluid shear stress to platelet receptors GpIb-IX-V and GpIb/IIIa, and hence inducing platelet aggregation.<sup>8</sup> Similarly, ULvWF forms have also been shown to be more effective than normal circulating vWF forms in supporting vWF attachment to a filter device.<sup>14</sup> They are not, however, a normal constituent of circulating plasma: the smaller circulating multimeric forms are thought to result from proteolytic degradation of ULvWF.

#### vWF proteolysis

Evidence for vWF proteolysis has been provided by a number of groups: vWF fragments with mobility corresponding to 189 kDa, 176 kDa, and 140 kDa are consistently detected in normal plasma in addition to the predominant 225 kDa subunit.<sup>15, 16</sup> It appears that circulating vWF multimers are originally composed of a varying number of intact 225 kDa subunit dimers but proteolysis causes the appearance of two predominant fragments of 140 kDa and 176 kDa. These originate as a consequence of cleavage of a single peptide bond between residues Tyr 842 and Met 843 of the mature subunit.<sup>17</sup> However, the precise mechanism by which this is achieved is unknown. It has been shown in vitro that normal plasma and cryosupernatant contain a substance that reversibly reduces the size of ULvWF multimers released from cultured endothelial cells into smaller multimeric forms. This reversible reaction appears to have the characteristics of a limited disulphide bond reductase.<sup>18</sup> Because ULvWF multimers are not a normal circulating constituent of plasma,

it was hypothesised that this process might be rendered irreversible by the subsequent proteolysis of partially unfolded vWF multimers.

Although vWF is stable in plasma, multimeric degradation results in vitro either at low salt concentrations, a process that is promoted by the presence of urea,<sup>19</sup> or after unfolding of vWF is induced by guanidine hydrochloride.<sup>20</sup> Under both conditions the fragments generated, of molecular weight 140 kDa and 170 kDa, are consistent with cleavage of the peptide bond between Tyr 842 and Met 843, suggesting that a physiological phenomenon is occurring. Using these models it has been shown that the vWF cleaving protease activity possesses several unusual properties. Unlike other known metalloenzymes, zinc ions are not required for full activity and barium rather than calcium ions activate the protease most efficiently. In addition, in contrast to acid lysosomal cathepsins the protease has an optimum pH of 8–10. Partial purification has linked the proteolytic activity to a high molecular weight protein (approximately 300 kDa) and it has therefore been proposed that the enzyme might be complexed with, for instance, a plasma protein inhibitor.<sup>19</sup>

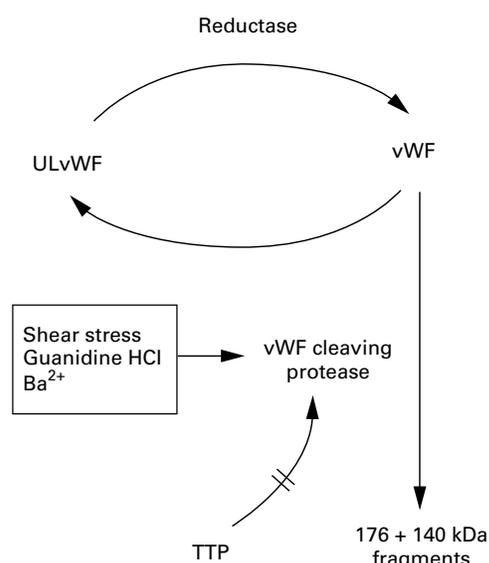
Although it has long been accepted that vWF function is affected by its multimeric size, the above studies also suggest that the conformation of the molecule is crucial in determining its proteolytic susceptibility. Exposure of tryptophan and/or tyrosine residues might be important because a shift in fluorescence to longer wavelengths is seen after incubation of vWF with guanidine HCl. A similar shift in fluorescence intensity of up to 4 nm can also be

observed when vWF is exposed to high shear stress. This is consistent with the finding that shear stress enhances the proteolysis of vWF in normal plasma<sup>21</sup> and has generated the proposed model of vWF proteolysis shown in fig 4.

#### vWF protease activity in TTP

Using the techniques discussed above, plasma derived from patients with both congenital and sporadic TTP has been tested in vitro for vWF cleaving protease activity because defective vWF multimeric processing could explain many of the above findings. Initially, a group of four patients with chronic relapsing TTP were shown to lack vWF metalloproteinase activity in the absence of inhibitor.<sup>22</sup> More recently, a total of 53 patients, 30 with TTP and 23 with HUS, were studied. Again, a lack of vWF cleaving activity was found in six individuals with familial TTP without evidence for a concomitant inhibitor. Of 24 with non-familial TTP, 20 had severe and four moderate protease deficiency. In only five of 15 such patients studied was there complete recovery of vWF cleaving activity with normalisation of the platelet count. Furthermore, in five of the remaining patients, protease activity was evident only after weeks or even months in clinical remission. An inhibitor was found in 20 subjects from this group and was of the IgG subclass in five of five cases tested. In contrast, of the 23 patients with HUS (familial and sporadic), 21 had normal values of vWF cleaving protease activity during the acute episode, whereas in two patients values were slightly reduced.<sup>23</sup> A similar phenomenon has been shown independently by Tsai: 39 samples from 37 patients with acute TTP all had severe deficiency of vWF cleaving protease. Inhibitory activity was detected in 26 of these 39 samples, again of the IgG subclass. Sixteen patients were studied during remission: vWF protease activity had normalised and there was no evidence of persisting inhibitory antibodies.<sup>24</sup> Thus, there is strong supporting clinical evidence that vWF cleaving protease activity is integral to the pathogenesis of TTP.

As a result of these findings, it has been proposed that acute single episode TTP might be an autoimmune disorder with a secondarily acquired functional deficiency of vWF cleaving protease. Why the metalloproteinase is selectively targeted and the cause of the transient or intermittent defect in immune regulation are unknown. If the protease were to be endothelial surface bound, protease specific antibody formation might result in endothelial cell apoptosis and hence endothelial disregulation. Certainly endothelial injury has long been considered by many to be the prime event in TTP, although the exact nature of the endothelial insult remains unclear. Thrombomodulin,<sup>25</sup> P-selectin (GMP-140),<sup>26,27</sup> tissue plasminogen activator (tPA),<sup>28</sup> and plasminogen activator inhibitor 1 (PAI-1)<sup>29</sup> are all raised, whereas tissue factor pathway inhibitor (TFPI)<sup>30</sup> is reduced, consistent with endothelial damage. These observations of endothelial activation, together with histological findings of intimal proliferation, luminal stenosis, and absent



**Figure 4** Proposed mechanism for proteolytic breakdown of von Willebrand factor (vWF). Ultra large forms of vWF (ULvWF) are reversibly converted in vitro to those forms normally found circulating in plasma by an activity in the cryosupernatant fraction of normal plasma with properties of a limited disulphide bond reductase. Under the conditions shown, a metalloproteinase further cleaves vWF forms into circulating 140 kDa and 176 kDa fragments, thus preventing reversible re-formation of ULvWF. This metalloproteinase activity has been reported to be absent in chronic relapsing thrombotic thrombocytopenic purpura (TTP), whereas it appears to be inhibited in single episode or intermittent adult TTP.

inflammatory changes, are consistent with apoptosis. There is some in vitro evidence for apoptosis of restricted lineage microvascular but not macrovascular endothelial cell lines induced by TTP plasma.<sup>31, 32</sup> This parallels the predilection for microvascular thrombosis within the renal and cerebral microvasculature seen clinically. Further work will be required to ascertain whether this mechanism is physiologically important.

Alternatively, should vWF cleaving protease be freely circulating, then autoantibody formation to the protease might be expected simply to result in circulating ULvWF multimers. However, as detailed above, preferential binding of such multimers to platelets under the conditions of high shear pressures that exist within the renal and cerebral microvasculature could explain the clinical features of the disease. Using flow cytometric techniques it has been shown that platelet bound vWF is raised in patients with TTP compared with normal adults.<sup>33</sup> Concordance between vWF and P-selectin expression was not a consistent finding, suggesting that the platelet bound vWF was derived from plasma rather than the platelet  $\alpha$  granules. This might explain the finding that although patients with single episode TTP classically have circulating ULvWF multimers, these can disappear as the episode continues or worsens.<sup>34</sup> Persistence of ULvWF multimers into clinical remission is predictive of subsequent relapse and might reflect the continuing presence of autoantibodies and consequent impaired ULvWF processing.

In contrast, chronic relapsing TTP appears to be caused by an absolute deficiency of protease activity that might be the result of a constitutional defect in its production, survival, or activity. Again, ULvWF multimers are often detected during remission, although relapses are associated with their disappearance. Because episodes occur at three to four week intervals, it has been suggested that the progressive accumulation of ULvWF multimers might periodically exceed a threshold value and precipitate platelet aggregation.

These hypotheses may, however, prove to be an oversimplification. A sibling of an index patient with congenital TTP has been identified with absent protease activity but, to date, no TTP or HUS like syndrome.<sup>25</sup> In addition, protease activity has not been shown to recover fully in all remission cases of adult onset TTP: whether this is the result of an interacting hitherto unrecognised cofactor or because subnormal values may be efficacious is yet to be elucidated. Indeed, the protease does appear to have a uniquely long half life: calculated as between two and four days in two patients with constitutional protease deficiency.<sup>35</sup> Moreover, metalloprotease activity was not significantly different from normal controls in eight patients studied with bone marrow transplant associated TTP.<sup>36</sup> This might suggest that there are alternative mechanisms mediating TTP or, in fact, that bone marrow transplant associated TTP is a distinct pathological entity. Certainly, it has long been recognised that contrary to

classic TTP, bone marrow transplant associated TTP rarely responds to plasma exchange procedures.

Despite these exceptions, the evidence at present suggests that vWF cleaving protease activity plays a central role in the pathogenesis of the major subtypes of TTP. Precise identification and purification of this vWF cleaving protease activity is now required to investigate its pathological role in greater depth. It is hoped that this will result in further advances in treatment and improve the prognosis of this potentially fatal disease.

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