Pathophysiology of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome

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Tables:	1
Figures:	1

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Summary

Thrombotic microangiopathies are rare disorders characterized by the concomitant occurrence of severe thrombocytopenia, microangiopathic hemolytic anemia, and a variable degree of ischemic end organ damage. The latter particularly affects the brain, the heart and the kidneys.

The primary forms, thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), although in their clinical presentation often overlapping, have distinctive pathophysiologies. TTP is the consequence of a severe ADAMTS13 deficiency, immune-mediated due to circulating autoantibodies (iTTP), or caused by mutations in the *ADAMTS13* gene (cTTP). HUS develops following an infection with Shiga-toxin producing bacteria (STEC-HUS), or as the result of excessive activation of the alternative pathway of the complement system because of mutations in genes of complement system proteins in atypical HUS (aHUS).

Key words

Thrombotic Thrombocytopenic Purpura; Hemolytic Uremic Syndrome; ADAMTS13; Alternative Complement Pathway; Shiga Toxin

Introduction

Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) are acute thrombotic microangiopathies (TMA), characterized by acute episodes of intravascular hemolysis, thrombocytopenia and microvascular thrombosis leading to end organ damage becoming apparent as acute kidney injury, cerebrovascular accidents or seizures, and myocardial infarction [1, 2]. The presence of fragmented erythrocytes (schistocytes) on the peripheral blood smear document the microangiopathic nature of hemolysis.

During the past two decades the knowledge on the pathophysiology of the primary TMAs, Shiga-toxin in typical or STEC-HUS, which follows a gastrointestinal infection with Shiga-toxin producing *Escherichia coli* (STEC), dysregulated and excessive complement activation in atypical HUS (aHUS), and lacking Von Willebrand factor (VWF) size regulation in the absence of ADAMTS13 in TTP have greatly advanced our understanding of these rare and often life-threatening diseases [1-5]. The most prevalent TMAs are STEC-HUS and TTP with an annual incidence of 2.17×10^{-6} (95% CI 2.00 - 2.34) of the latter [6].

The concomitant presence of thrombocytopenia and microangiopathic hemolytic anemia is non-specific and can also be observed in a number of other diseases and conditions such as preeclampsia / HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome in pregnancy, after stem cell transplantation, in disseminated cancer, or disseminated intravascular coagulation, associated with malignant hypertension, HIV infection, the catastrophic antiphospholipid syndrome and other autoimmune disorders, or may be induced by certain drugs [1, 2]. TMAs associated with underlying or coexisting conditions are considered secondary TMAs. It should be noted however, that a number of these conditions have been documented as triggers of a presenting episode of TTP or aHUS [1, 7-11]. Appropriate laboratory work-up, including ADAMTS13 testing, is consequently warranted even in apparently clear secondary TMAs.

Here we review the current understanding of the pathophysiology of the primary TMAs with an outlook on how this knowledge impacts patient management and treatment.

Thrombotic thrombocytopenic purpura

Key components in the pathophysiology of TTP are Von Willebrand factor (VWF) and its primary size regulator, ADAMTS13 [1, 3]. The *ADAMTS13* gene covers ~37 kilobases on chromosome 9q34 [12] and encodes a multidomain protein of 1427 amino acid residues (Figure). Although low ADAMTS13 mRNA levels have been detected in many tissues such as brain, heart, kidney, placenta, muscle, testis, ovary, and platelets, only hepatic stellate cells, podocytes and renal tubular epithelial cells, platelets, and endothelial cells (EC) have been shown to produce biologically active ADAMTS13 protein (reviewed in [1]).

VWF is synthesized by megakaryocytes and ECs and stored in the form of ultra-large multimers in α-granules of platelets, and in Weibel-Palade bodies of ECs [13]. Upon vascular injury or EC activation ultra-large VWF multimers are released or secreted into the circulation but remain anchored on the vessel wall, where they promote platelet adhesion and aggregation. Shear forces of the flowing blood unfold coiled VWF, exposing cryptic platelet binding sites as well as the ADAMTS13 cleavage site in the VWF A2 domain. Reciprocally induced conformational changes in both VWF and ADAMTS13 result in regulated proteolysis of the ultra-large VWF multimers into smaller, less sticky forms [14-16]. During this process, the interaction between the ADAMTS13 CUB and spacer domains is loosened and functional exosites in the ADAMTS13 spacer domain become exposed (Figure, panel B) [14-16]. Although, this likely optimizes VWF size regulation under shear conditions, it might also render ADAMTS13 susceptible to immune recognition. In the absence of ADAMTS13 ultra-large VWF multimers persist and spontaneously bind platelets leading to VWF-rich thrombi occluding the microcirculation, the pathological-anatomical hallmark of TTP.

Apart from ADAMTS13, a number of leukocyte proteases, such as elastase, proteinase 3, cathepsin G and matrix metalloprotease 9 (MMP9) as well as the coagulation factors thrombin and plasmin are able to cleave VWF at sites near or at Tyr1605 – Met1606, the ADAMTS13 cleavage site in the VWF A2 domain [17-19].

Today, two forms of TTP are distinguished. In acquired or immune-mediated TTP (iTTP), severe ADAMTS13 deficiency is the result of circulating antibodies inhibiting ADAMTS13

activity or increasing ADAMTS13 clearance. In congenital TTP (cTTP, also known as Upshaw-Schulman syndrome [20, 21]), severe ADAMTS13 deficiency is caused by homozygous or double heterozygous *ADAMTS13* mutations.

Immune-mediated TTP

Twenty years ago, in 1997 the link between TTP and a severe ADAMTS13 deficiency was established in four patients [22], and soon thereafter confirmed in additional patients [23, 24], where already the acquired nature of the ADAMTS13 deficiency as a consequence of anti-ADAMTS13 IgG in the majority of patients was demonstrated [23, 24].

Initial ADAMTS13 assays were cumbersome and had detection limits around 5-6.25% of the normal [25]. Nowadays, assays using a VWF-peptide substrate, instead of full-length VWF are widely available, robust and easy to perform. The sensitivity of many assays has been increased and detection limits as low as 0.5 to 1% are achieved [26-28]. Nevertheless, the clinically relevant threshold in iTTP is usually set at 10% of the normal, as an ADAMTS13 activity <10% at presentation with the acute episode seemed best to distinguish survivors of a first acute TMA episode at risk of relapse [29], and was used to define TTP [30]. Recently, two groups reported that patients having an ADAMTS13 activity of 10-20% in the presence of anti-ADAMTS13 antibodies had a similar outcome as iTTP patients with a severe ADAMTS13 deficiency [31, 32].

Antibodies to ADAMTS13

Antibodies to ADAMTS13 are classified into inhibitory, assessed with Bethesda-like assays, and non-inhibitory, substantiated usually by enzyme-linked immunosorbent assays (ELISA). Virtually all patients presenting with an acute iTTP episode have circulating anti-ADAMTS13 antibodies. While in most patients' plasma strong functional inhibitors are present, 10-15% of iTTP patients have only non-inhibitory ADAMTS13 antibodies, which . are also detected in plasma of ~10% of healthy controls, as well as in immunoglobulin preparations [33]. Both types of antibodies increase ADAMTS13 clearance [34-36].

Anti-ADAMTS13 antibodies are mainly of IgG isotype [34, 35, 37-40], but in up to 20% patients also IgA and IgM anti-ADAMTS13 antibodies have been observed [37, 39, 40]. The most abundant IgG subclasses are IgG_4 and IgG_1 [38-40]. Distinction between isotypes and IgG subclasses may have some prognostic value, as the presence of IgA and/or IgG_1 at presentation with an acute iTTP episode was associated with a higher death rate [37, 38, 40], while high levels of IgG_4 were found to be linked with an increased risk of relapse, and in relapsed cases often the only isotype present [38].

The primary epitope recognized by anti-ADAMTS13 antibodies is located in the spacer domain [41-43] (Figure, panel C upper part). Two thirds of patients have antibodies reacting with epitopes in other ADAMTS13 domains in addition, underlining the polyclonal nature of the autoimmune response in iTTP [36, 41-43].

Epitope fine mapping in the ADAMTS13 spacer domain revealed that five amino acid residues, the positively charged Arg568 and Arg660, as well as Phe592, Tyr661, and Tyr665 constituted the principal antigenic surface of the majority of inhibitory ADAMTS13 antibodies [39, 44-47]. When ADAMTS13 adopts a folded conformation these amino acid residues are shielded by the two CUB domains, but likely protrude in the open conformation, a hypothesis supported by the crystal structure of the N-terminal ADAMTS13 domains [48]. Introduction of mutations replacing the amino acids in the primary antigenic surface resulted in an ADAMTS13 resistant to inhibition by autoantibodies [49].

New data of ADAMTS13 conformation on autoantibody binding was presented at the ISTH 2017 congress in Berlin. Underwood *et al*. showed that the majority of iTTP patients had ADAMTS13 antibodies recognizing both the closed and open ADAMTS13 conformation, the latter however, was the only conformation recognized in 3/17 (16.7%) patients [50]. Despite a severe ADAMTS13 deficiency, a considerable number of iTTP patients have detectable ADAMTS13 antigen, primarily present in form of circulating immune complexes [11, 31, 51, 52]. Making use of a mouse monoclonal antibody recognizing ADAMTS13 exclusively in its open conformation, Roose *et al*. [53] demonstrated that in healthy individuals, in patients suffering from HUS or sepsis (where ADAMTS13 activity is usually only mildly or moderately

reduced) ADAMTS13 was present in a closed conformation, while in virtually all iTTP patients investigated, irrespective of the level of ADAMTS13 antigen, ADAMTS13 displayed an open conformation. The reason for the observed open conformation in iTTP needs to be further explored.

Cloning of anti-ADAMTS13 antibodies of iTTP patients shed light on their autoreactive B cell repertoire [47, 54-56]. VH1-69 and VH1-3 heavy chain gene usage is commonly observed and documented somatic mutation rates are compatible with affinity maturation of the ADAMTS13 autoantibodies [47, 54, 55]. An excess of negatively charged amino acids in the complementarity-determining region 3 (CDR3), the primary antigen binding site of an antibody, mirrors the positive charge of the ADAMTS13 antigenic surface [47]. Longitudinal investigations in relapsing iTTP patients demonstrated functional maturation, from non-inhibitory to inhibitory anti-ADAMTS13 antibodies, and/or changes in epitope recognition over time, suggesting a continuous development and shaping of the autoimmune response to ADAMTS13 in iTTP [11, 36].

Role of T-cells

As yet, little is known on the contribution of T cells to the pathophysiology of iTTP. The T-cell compartment can be divided into two main cell entities, cytotoxic $CD8^+$T-cells and $CD4^+$T-cells, which regulate antibody production by interacting with B-cells. The IgG isotype of ADAMTS13 antibodies, as well as the documented somatic hypermutation in characterized human ADAMTS13 antibodies [47, 54-56], supports the involvement of autoreactive $CD4^+$T-cells in the pathogenesis of iTTP. The *HLA-DRB1*11* allele, identified as a risk factor for the development of iTTP [57-59], encodes an MHC class II molecule particularly suitable to present specific ADAMTS13 peptides to $CD4^+$T-cells [60-62]. The highest presentation efficiency was observed for CUB2 domain-derived peptides [60]. In silico prediction of candidate T-cell epitopes of ADAMTS13 and subsequent wet lab experiments identified a slightly different CUB1 peptide, $ADAMTS13^{1239-1253}$ as the single immune-dominant HLA-DR1-restricted $CD4^+$T-cell epitope [62]. For all these ADAMTS13 CUB peptides, autoreactive

$CD4^+$T-cells were demonstrated in iTTP patients [61, 62].

Possible triggers and risk factors of autoimmunity to ADAMTS13

The causes of loss of self-tolerance and the initiation of an autoimmune response to ADAMTS13 are still poorly understood.

Infections. Many patients report a mild infection (upper respiratory tract or urogenital) in the week(s) preceding the acute event. The occurrence of iTTP with severe immune-mediated ADAMTS13 deficiency following an infection with influenza viruses has been documented (reviewed in [1]). Of note, the immune system frequently uses the IGHV1-69 heavy chain to develop antibodies to influenza, particularly for neutralizing antibodies to the influenza hemagglutinins [63, 64]. In four different patient cohorts with established STEC-HUS, a few patients were found to have bona fide iTTP with severe immune-mediated ADAMTS13 deficiency at the same time [1]. A certain role for lipopolysaccharides (LPS), components of the outer-membrane of Gram-negative bacteria such as *E. coli*, is at least implied by the observation of a strong linkage with the gene for acyloxyacyl hydrolase (*AOAH*) in iTTP patients, an enzyme involved in LPS inactivation [65]. Whether infections with Stx producing *E. coli* elicited an autoimmune response to ADAMTS13, or whether the *STEC* infection represented the missing trigger or second hit to set off an overt acute TTP episode in patients with a preexisting autoimmune response to ADAMTS13 is unknown.

Genetic factors. There is evidence for a certain heritable predisposition for the development of ADAMTS13 antibodies and iTTP. First to mention is the disproportionate representation of certain ethnicities within iTTP cohorts compared to the respective resident populations, with African-Americans or African-Caribbean more frequently suffering from iTTP than Caucasians [1, 6].

Likewise the familial occurrence of acute episodes of iTTP with documented severe immune-mediated ADAMTS13 deficiency underscores a genetic predisposition for iTTP. We know of four as yet unpublished families with more than one iTTP patient. Furthermore, identical twin sisters suffering from iTTP episode more than one year apart [66] as well as a second family

with two affected sisters [67] have been reported. Of note, none of these four women from two separate iTTP families carried the *HLA-DRB1*11* allele identified as risk factor to develop iTTP [57-59, 65]). Documentation of *ADAMTS13* mutations, causative for cTTP, in heterozygous state in a number of iTTP patients (accounting for 11% and 9.6% of patients in the respective iTTP cohorts) completes the picture [68, 69].

Other factors. Children (before puberty) rarely develop iTTP, while women and blacks are more frequently affected than men or non-blacks [1, 2, 6]. The population affected by iTTP shares thus several characteristics with other autoimmune disorders, especially systemic lupus erythematosus (SLE), which may clinically present as TMA [1, 2, 6, 29] and differential diagnosis of thrombocytopenia in SLE includes iTTP. Anti-nuclear antibodies (ANA), typical though not specific for SLE, have been reported to be present in the majority of iTTP patients at presentation with the first acute episode [1, 2]. SLE can precede iTTP or develop in survivors [1, 2, 6, 29, 35, 70], where increased prevalence has been demonstrated [70].

Lessons learned from clinical presentation and follow-up of survivors of a first iTTP episode.

The introduction of plasma exchange with replacement of fresh frozen plasma – removing antibodies to ADAMTS13, VWF and cytokines, and replenishing ADAMTS13 at the same time - lead to large numbers of TTP survivors with new problems emerging. The major problem is the risk of relapse, which is almost exclusively conferred to patients having a severe ADAMTS13 deficiency at presentation with the acute episode [29], and is highest in patients with persistence or reappearance of a severe ADAMTS13 deficiency in remission [71, 72]. ADAMTS13 activity is now more and more used as a biomarker in follow-up of patients as well as to initiate preemptive treatment when ADAMTS13 activity is decreasing below 10-15% [2]. Recently, Page *et al* reported on the follow-up of 57 iTTP patients for up to 9 years [72]. In seven of 17 patients (41%) who had at least one ADAMTS13 activity <10% during follow-up a spontaneous recovery of ADAMTS13 activity to normal levels was observed, most patients however, had fluctuating ADAMTS13 activity levels over time.

Although spontaneous ADAMTS13 recovery is possible, roughly 60% of patients with a severe ADAMTS13 deficiency in remission experienced at least one iTTP relapse [72] and regular follow-up of iTTP survivors in remission including ADAMTS13 monitoring may be useful to predict relapses.

Despite the risk of relapse, until recently, we tended to refer to survivors as status post iTTP. The observed long-term morbidities in this patient group (arterial hypertension, major depression, neurocognitive deficits and, particularly the unexplained, reduced life-expectancy) [70] hint at a much higher chronicity of iTTP than had been anticipated. The presence of circulating ADAMTS13 immune complexes even years after an acute iTTP episode [52, 73] also suggests a chronic ongoing disease and challenges the concept of remission in iTTP.

The introduction of rituximab, a humanized anti-CD20 monoclonal antibody originally developed to treat $CD20^{+}$B-cell neoplasia, into iTTP therapy has greatly reduced the risk of relapse (for a review see [1, 2]). Investigation of the splenic B-cell repertoire of relapsing iTTP patients treated with or without rituximab, in whom splenectomy was finally performed as another measure to reduce the risk of relapse, revealed that the spleen is a reservoir of a considerable number of ADAMTS13 specific B-cells, including CD20-negative plasmablasts and plasma cells [47].

Congenital TTP

Although the true prevalence of Upshaw-Schulman syndrome (OMIM #274150) is unknown, often a number of 1 in one million is put forward. Estimates based on identified cases in defined regions suggest that the point-prevalence might lie in the range of 0.4 to 16.7 per million [10, 28]. The high estimate for Central Norway is matched by a considerable allelic frequency of the two most prevalent *ADAMTS13* mutations in this population, *c.4143_4144dupA* and p.R1060W of 0.04 – 0.33%, and 0.3-1%, respectively [28]. The lower point-prevalence estimates might be too conservative, as increasing numbers of cTTP patients with adult disease-onset are identified [8, 9].

Congenital ADAMTS13 deficiency is an autosomal recessive feature and thus the result of bi-allelic mutations. So far more than 150 different causative *ADAMTS13* mutations spreading over all ADAMTS13 protein domains have been identified (reviewed in [74]). The majority of mutations are missense mutations (~62%), followed by deletions and insertions (~19%), nonsense (~10.5%) and splice site (~8.5%) mutations (Figure, panel C lower part).

Although a monogenic disorder, the clinical presentation of cTTP is often variable, even among patients carrying the same mutations, as well as among affected siblings. Overall, age at onset and diagnosis shows a seemingly dichotomous distribution with about half of patients presenting within their first 2-5 years of life and a second peak in early adulthood, specifically during pregnancy. Among women with a first TTP episode during their first pregnancy the frequency of cTTP was 24% (10/42 women) and 66% (23/35 women), respectively [8, 9]. Remarkable is the prevalence of the *ADAMTS13* mutation p.R1060W in this special group of cTTP patients, 8/10 (80%, French cohort) and 17/23 (74%, UK cohort) of patients, respectively, were either compound heterozygous or homozygous carriers of this mutation [8, 9]. This *ADAMTS13* mutation is associated with residual ADAMTS13 activity, 3-6% in cTTP patients with a single p.R1060W allele, and 5-12% in homozygous carriers [27]. The case histories of adult-onset cTTP demonstrated that many patients had exchange transfusions in their first days of life [10, 28], questioning their genuine adult-onset.

In search of factors influencing the variable clinical course in cTTP, residual ADAMTS13 activity <3% was found to be associated with an early disease onset (<18 years of age), an annual event rate >1, and a necessity for prophylactic plasma therapy [27]. The observed clinical variability, however, cannot be explained by differences in residual ADAMTS13 activity alone, as very variable disease courses have been documented in a large number of cTTP patients homozygous for the *c.4143_4144dupA* mutation, having typically an ADAMTS13 activity <1% of the normal [28, 75].

While in iTTP ADAMTS13 seems to be a partner in the dysregulated immune response leading to the development of autoantibodies to ADAMTS13, allo-antibodies to ADAMTS13 have only occasionally been observed in cTTP patients on regular prophylactic plasma

infusions [1, 10]. Except for two cases in whom low titer (<5 BU/ml), functional ADAMTS13 inhibitors were observed, the allo-antibodies in the other cases were most often non-inhibitory IgG fluctuating in titer levels, and didn't seem to interfere with ADAMTS13 recovery or plasma half-life [1, 10].

The *ADAMTS13* gene contains a number of non-synonymous sequence variants. Of particular interest are p.P618A and p.A732V which in combination strongly reduce ADAMTS13 antigen and activity levels when expressed in HEK293 cells (each ~10% of that of wild-type ADAMTS13) [76]. Introduction of the p.R7W and p.Q448E variants on the same allele acted positively on ADAMTS13 secretion (raised to ~65% of wild-type) but were unable to fully rescue the severely reduced activity conferred by p.P618A (ADAMTS13 activity of p.WEAV ~40% of wild-type ADAMTS13). In a number of studies, patients with an ADAMTS13 activity <10% of the normal in the absence of ADAMTS13 antibodies and only one documented *ADAMTS13* mutation but carrying the p.WEAV allele in addition were considered to have cTTP [8, 9].

Shiga-toxin associated HUS (STEC-HUS)

STEC-HUS is the most common cause of acute kidney injury in children <5 years of age, and rare in adults. Most cases are sporadic, and larger outbreaks, such as the West of Scotland or the 2011 German outbreak attracted much publicity [5, 77-79].

Most commonly implicated are *E. coli* subtypes that have acquired a bacteriophage enabling the production of Stx (*E. coli* serotype O157:H7 accounts for ~ 70% of cases in the Western world, but other strains, i.e. O118:H2, O111:H or O104:H4 are also involved [5, 77, 80]). An aggressive, at the time unknown STEC variant, *E. coli* O104:H4, which combined characteristics of typical enteroaggregative *E. coli* with the ability to produce Stx, was responsible for the German 2011 outbreak with roughly 4000 affected patients of whom 22% developed HUS [78, 79]. The majority of these STEC-HUS patients were adults (88%), many presented with neurological involvement and 50 died [78, 79]. These numbers are clearly

higher than seen in prior outbreaks, where infections usually were mild and self-limiting, and only 10-15% of affected patients subsequently developed STEC-HUS [77].

The systemic illness is caused by Stx-mediated injury to the vascular endothelium and a generalized inflammatory response. Stx consists of five glycolipid-binding B subunits and one enzymatically active A subunit, that inhibits protein synthesis by cleaving 28S ribosomal RNA eventually leading to apoptotic cell death [80]. After colonic infection with enterohemorrhagic bacteria, Stx is absorbed across the intestinal epithelium into the blood stream, where it binds to and is internalized by globotriaosylceramide, also known as Gb3, CD77 or P^k blood group antigen [80]. Gb3 is a ganglioside and a non-protein receptor on ECs, predominately of small vessels of the gut, the kidneys, where it is strongly expressed on glomerular ECs, and the brain, leading to bloody diarrhea, renal insufficiency and neurological complications [80, 81]. In addition, there is evidence that Stx can activate the complement system possibly explaining lower C3 and elevated soluble terminal complement complex (sC5b-9) levels seen in some STEC-HUS patients [82]. In addition, Stx can reduce the expression of the GPI-anchored complement regulator CD59 on human tubular epithelial and glomerular ECs, inhibit factor H, the most important fluid phase complement regulator, and induce a procoagulant state by increasing the expression of tissue factor on ECs and/or by the activation of platelets [80].

Atypical HUS

Complement-mediated or aHUS is the consequence of excessive activation of the alternative pathway (AP) of complement because of mutations in complement regulators or complement factors (heritable, though incomplete penetrance), or autoantibodies against factor H (acquired aHUS with strong genetic linkage) [4, 5]. Although the clinical features may resemble those of STEC-HUS, prognosis is more reserved and recurrence is frequent. The prevalence of aHUS is unknown but is thought to account for <5% of all HUS cases [4, 5].

Complement is part of the innate immune system and enhances (or complements) the ability of antibodies and phagocytes (granulocytes, monocytes and macrophages) to clear

pathogens, and damaged or dead cells. Complement is activated via three pathways which all lead to target elimination by phagocytosis and/or direct lysis. The three pathways are: *i*) the classical pathway, initiated by binding of C1q to IgG or IgM bound on targets. *ii*) the lectin pathway initiated by the binding of mannose-binding lectin or ficolin to certain sugar moieties on targets; and finally *iii*) the alternative pathway (AP), which is distinct from the two other pathways as it rests on constant and spontaneous low-level activation leading to deposition of C3b on virtually all cell surfaces in contact with plasma [5]. If these C3b deposits are not cleared or inactivated on the cell surface, they form together with complement factor B the C3 convertase (C3bBb) resulting in the amplification of the complement system activation.

The complement system is tightly regulated by a number of cell-membrane bound regulators, CD35 (complement receptor 1, CR1; not expressed on platelets), CD46 (membrane-cofactor protein; MCP, not expressed on red blood cells), CD55 (decay accelerating factor, DAF) and CD59, as well as by in plasma circulating complement regulators, factors I and H. Under steady state conditions regulation exceeds activation.

Today, in 50-70% of aHUS cases causative mutations in genes of the complement system or associated proteins are identified, both in sporadic (comprises ~80%) and in familial cases (Table) [4, 5]. These defects are loss-of-function mutations in *CFH*, *CFI*, *MCP*, or *THBD* (the gene encoding thrombomodulin, which enhances in the presence of factor H factor I-mediated inactivation of C3b, and is a cofactor of thrombin in the generation of TAFIa, which inactivates the anaphylatoxins C3a and C5a) leading to a defective AP regulation. Gain-of-function mutations have been described in complement factors *C3* and *factor B* genes, the two components of the AP C3 convertase (Table). Incomplete phenotypic penetrance (close to 50%) is observed in many mutation carriers and families, where carriers of the same mutation may also show different symptoms and time points of disease onset. The concomitant presence of multiple different risk factors and/or complement mutations is fairly common [83-85] and it is thought that many of the reported aHUS-associated gene variants predispose rather than cause the disease [83, 85].

In 5% to 10% of aHUS acquired complement dysregulation is present due to anti-factor H antibodies, which bind to epitopes in the C-terminal short consensus repeats (SCR) 19 and 20 of factor H and have functional consequences similar to the prototypical mutations in this factor H region [4, 5, 85]. This autoimmune form of aHUS has a high risk of relapse and end stage renal disease, and is in the majority of cases associated with bi-allelic deletions of the *CFH*-related gene 1 (*CFHR1*) and/or *CFHR3* [4, 5, 85].

Effective inhibition of the complement system can be achieved with eculizumab, a monoclonal antibody blocking the activation of C5 to C5a and C5b and thus the generation of the terminal complement complex C5b-9. Eculizumab has been proven very effective in reverting the clinical presentation in aHUS patients with long standing disease courses [86], though indefinite treatment may not be required in all aHUS patients, as except *CFH* mutation carriers, most patients don't relapse once the trigger of the acute episode is removed or taken care of [87, 88].

There have been a few patients described in whom a documented STEC infection acted as trigger and unmasked thus far latent complement defects in patients subsequently noted to have bona-fide aHUS (reviewed in [89]).

Overlap or common terminal pathway to overt TMA

Up to here, we have presented the pathophysiology of TTP and HUS in a dichotomous way – VWF or ADAMTS13 on one side, infection and excessive activation of the AP of complement on the other side. However, evidence of functional interactions between the VWF-platelet axis and complement activation is steadily accumulating.

Contribution of complement mutations to the phenotypic presentation in cTTP was first described by Noris *et al.* [90] who reported a cTTP family with three affected siblings, two sisters with phenotypically distinct clinical pictures with kidney failure as leading sign in one of them, and an asymptomatic brother. Besides the compound heterozygous *ADAMTS13* mutations (one conferring residual activity) present in all three siblings, merely the sister with the renal involvement carried in addition a heterozygous *CFH* mutation, previously found in

aHUS. In a small case series of 32 cTTP patients, 13 with and 19 without renal involvement, Fan *et al*. [91] observed the same prevalence of missense sequence variants known to confer an increased risk for aHUS in complement genes in both patient groups. However, in one of the cTTP patients with renal involvement a novel *C3* mutation, p.K155Q located in a region of C3 where aHUS-associated mutations cluster, was identified.

Vice versa, heterozygous *ADAMTS13* mutations or sequence variants were identified in a small aHUS cohort [92], where many patients displayed moderately to mildly reduced ADAMTS13 activity at presentation with the acute disease episode. In another report 3/17 patients had heterozygous *ADAMTS13* mutations or sequence variants in addition to complement mutations [88].

Obligatory heterozygous *ADAMTS13* mutation carriers are healthy, have typically an ADAMTS13 activity of ~50%, and don't experience TTP episodes. However, mild thrombocytopenia has been documented in some of them during pregnancy or infections [1], conditions known to be associated with increased VWF levels and thus possible increased demand on VWF size regulation by ADAMTS13. Pregnancy is also a recognized trigger of aHUS [7]. Together these observations support an interplay of hemostasis and the complement pathway with a possible dosing effect, the more markers present the higher the risk of overt TMA.

In vitro, endothelial-cell anchored ultra-large VWF multimers are capable to bind C3b, the active form of complement factor C3, which subsequently assembles the C3 convertase (C3bBb) and C5 convertase (C3bBbC3b) [93]. This occurs particularly in the absence of ADAMTS13 [94, 95], and is halted or reverted by the addition of ADAMTS13 [95]. During acute TTP episodes complement is activated, however, to a lesser extent than in aHUS [96, 97]. Although most probably a secondary phenomenon, this complement activation in iTTP will enhance platelet activation, cause further EC damage with release of additional ultra-large VWF multimers and fosters the process of thrombotic microangiopathy. Microangiopathic hemolysis is common to both TTP and aHUS. Free heme triggers AP complement activation leading to C3b deposits in EC, which is paralleled by a decreased

expression of MCP and CD55. Moreover, heme is able to induce VWF secretion from Weibel-Palade bodies and expression of P-selectin, a known C3b-binding protein, on ECs [98].

Another shared feature are elevated nucleosome levels, which are detected at presentation in the majority of patients with an acute TTP or HUS episode [99]. The authors of the accompanying editorial suggested that nucleosomes and neutrophil extracellular traps (NETS) might constitute a common terminal pathway to overt TMA in patients at risk [100].

Outlook

The new pathophysiological insights into TTP and HUS have already had a tremendous impact on treatment helped to wear off some of the grimness of these rare diseases. Ever growing numbers of survivors reveal new questions. How is remission achieved in iTTP, in particular what keeps the dysregulated immune response in some patients in check, while others frequently relapse often despite immunosuppressive therapy. Understanding the role of ADAMTS13 and its conformational changes in this process [50, 53] may be essential. Similarities in the immune dysregulation observed in iTTP and SLE point to shared pathophysiologies that lead to the loss of tolerance to self-antigens. Given the infectious or inflammatory triggers often reported by patients preceding disease onset, epigenetic changes in gene expression and posttranslational modifications related to environmental influences should be further explored.

The incomplete penetrance in aHUS as well as individual factors fostering the progression from infection with Stx-producing bacteria to STEC-HUS are so far poorly understood. Positive confirmation of aHUS diagnosis with appropriate biomarkers might help to identify patients who would benefit from short- or long-term of anti-complement therapy. With the prospect of ever growing patient cohorts, the possibility of employing human phenotype ontology systems, of whole exome and genome sequencing, new developments in proteomics and other –omics, and data sharing, deeper insights and new interactions between the once so distinct TMA forms are likely to emerge in the future, further linking hemostasis and the complement system.

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Table

Complement genes and proteins identified in atypical HUS.

Prevalence and prognosis (before the introduction of complement inhibitor Eculizumab) are adapted from Afshar-Kharghan. and Jokiranta [4, 5].

Mutated gene	Localization	Prevalence	ESRD / death*
Factor H	plasma	24—28%	70-80%
CFHR1/3 deletion w. anti-FH antibodies	plasma	3-10%	30-70%
Factor I	plasma	4-8%	60-70%
MCP (CD46)	membrane	5-9%	<20%
Thrombomodulin (THBD)	plasma & membrane	0-5%	50-60%
Factor B	plasma	0-4%	70%
C3	plasma	2-8%	60-70%
Diacylglycerol kinase ε	plasma	0-3%	46
None identified		30-48%	50%

* Abbreviations: ESRD End stage renal disease; CFHR Complement factor H related; MCP Membrane cofactor protein

Legend to figure

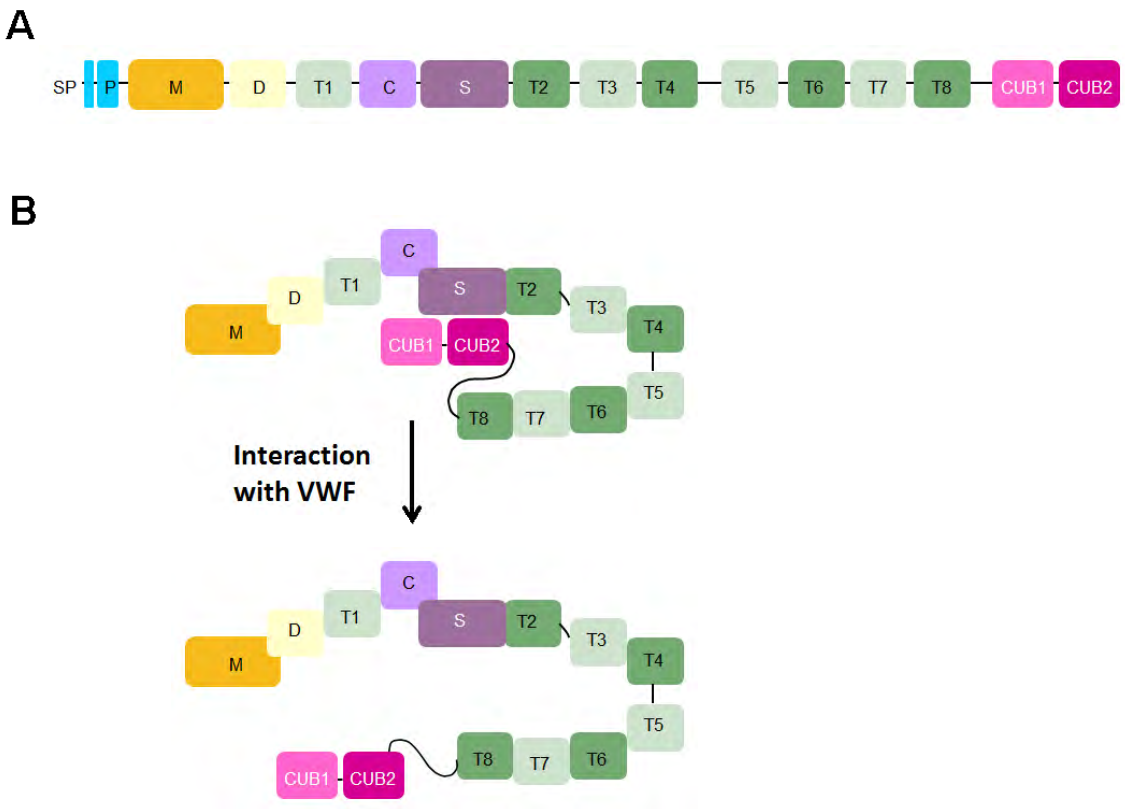
Domain structure and conformation of ADAMTS13

Panel A. The *ADAMTS13* gene is located on chromosome 9q34, contains 29 exons and encodes a multi-domain protein of 1427 amino acid residues. The protein domain structure consists of a signal (SP) and a pro-peptide (P), which are cleaved of before the active protein is secreted, a metalloprotease domain (M), a disintegrin domain (D), a first thrombospondin type 1 repeat (T1), a cysteine-rich (C) and a spacer (S) domain, followed by another seven thrombospondin type 1 repeats (T2-T8) and two CUB domains. The same color code is used as in Kremer Hovinga *et al* [1].

Panel B. The new concept of a closed and open ADAMTS13 conformation is shown according to ideas of South *et al.* [14], Muia *et al.* [15], and Roose *et al*. presented at the ISTH 2017 congress in Berlin [53]. In the closed conformation the CUB domains interact with the spacer domain, thereby concealing the principal epitope of anti-ADAMTS13 autoantibodies present in plasma of the majority of iTTP patients. Upon binding to Von Willebrand factor a conformational change and activation of ADAMTS13 takes place resulting in an open conformation where the CUB domains no longer shield the spacer domain.

Panel C. Immune-mediated TTP (upper row): Frequency of recognition of specific ADADMTS13 domains by anti-ADAMTS13 antibodies (Y) in different studies [36, 41-43]. Lower row, congenital TTP: Majority of identified causative mutations in patients with congenital ADAMTS13 deficiency.

Figure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Interaction
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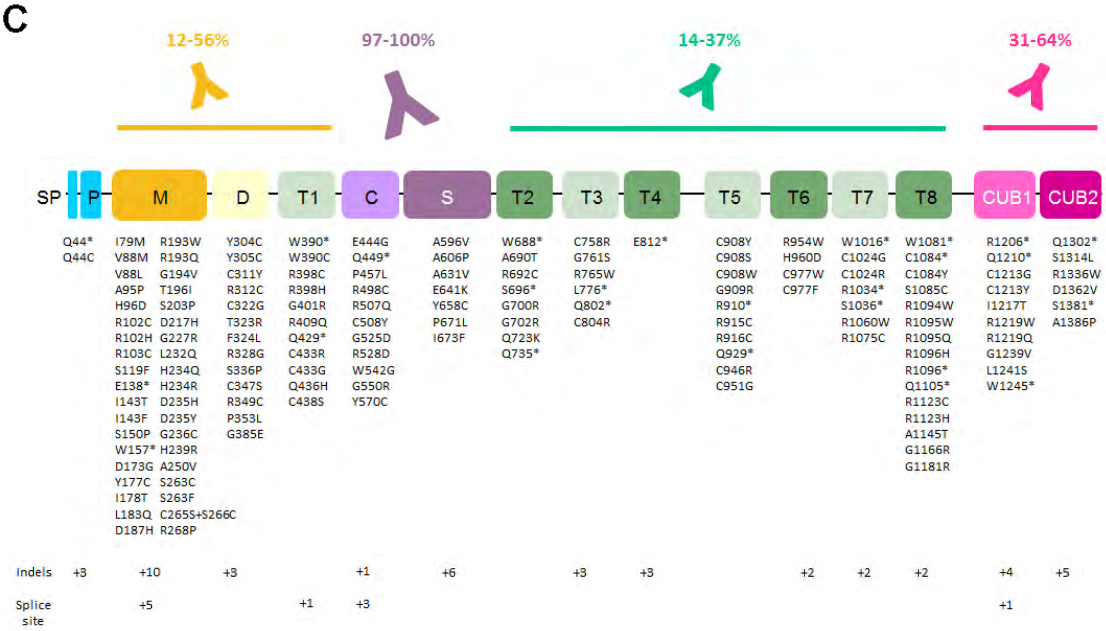
C


12-56%
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I79M
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A95P
H96D
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R103C
S119F
E138*
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I143F
S150P
W157*
D173G
Y177C
I178T
L183Q
D187H
R193W
R193Q
G194V
T196I
S203P
D217H
G227R
L232Q
H234Q
H234R
D235H
D235Y
G236C
H239R
A250V
S263C
S263F
C265S+S266C
R268P
Y304C
Y305C
C311Y
R312C
C322G
T323R
F324L
R328G
S336P
C347S
R349C
P353L
G385E
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R398H
G401R
R409Q
Q429*
C433R
C433G
Q436H
C438S
E444G
Q449*
P457L
R498C
R507Q
C508Y
G525D
R528D
W542G
G550R
Y570C
A596V
A606P
A631V
E641K
Y658C
P671L
I673F
W688*
A690T
R692C
S696*
G700R
G702R
Q723K
Q735*
C758R
G761S
R765W
L776*
Q802*
C804R
E812*
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R910*
R915C
R916C
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H960D
C977W
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L1241S
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D1362V
S1381*
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