Thrombotic Microangiopathies: Towards a Pathophysiology-Based Classification

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Abstract: Thrombotic microangiopathies (TMA) encompass various diseases characterized by a microangiopathic hemolytic anemia, platelet clumping, and organ failure of variable severity. Thrombotic thrombocytopenic purpura (TTP) is a particularly severe form of TMA characterized by systemic organ failure which results from a severe defect in ADAMTS13, a plasma enzyme specifically involved in the cleavage of highly hemostatic unusually large (UL) von Willebrand factor (VWF) multimers into smaller and less adhesive VWF forms. Failure to degrade these UL-VWF multimers leads to excessive platelet aggregates and capillary occlusion. ADAMTS13 deficiency results from bi-allelic mutations in hereditary TTP, whereas in acquired forms it results from autoantibodies that alter the protein function. Patients with acquired idiopathic TTP have a trend to develop autoimmunity, since a clinical context of autoimmunity may be found in 30% of cases. Moreover, the remarkable efficiency of monoclonal antibodies directed against CD20 antigen of B lymphocytes in refractory or chronic relapsing forms provides an additional indirect argument to consider acquired TTP as an autoimmune disease.

Hemolytic uremic syndrome (HUS) is characterized prominently by a renal failure. In most cases, HUS is caused by enterohemorrhagic Escherichia coli (diarrhea-positive HUS). Diarrhea-negative HUS, termed atypical HUS, was associated with a dysfunction in complement pathway involving mutations in factor H, factor I, CD46/MCP, factor B and C3 components.

The major improvement in our understanding of TMA pathophysiology allows now a more accurate molecular classification of TMA syndromes, which opens fascinating perspectives of targeted therapies in the forthcoming years.

Key Words: Thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, thrombotic microangiopathies, thrombocytopenia, ADAMTS13, autoimmune disease, complement.

INTRODUCTION

Thrombotic microangiopathies (TMA) encompass a heterogeneous group of disorders characterized by the association of a microangiopathic hemolytic anemia (Fig. 1A) with peripheral thrombocytopenia and organ injury of variable severity, that result from microthrombi in capillaries and arterioles (Fig. 1B). These diseases can affect people of all classes of age, although some forms are more frequently observed in children, while others are more likely encountered in adult population. Distinct subsets of TMA can be distinguished depending on clinical presentation and physiopathological mechanisms. In 1924, Moschcowitz described thrombotic thrombocytopenic purpura (TTP) as an acute febrile plechroic anemia with hyaline thrombi in the terminal arterioles and capillaries of the brain, heart, pancreas, spleen, kidney and adrenal gland [1]. In hemolytic uremic syndrome (HUS), severe acute renal failure with bilateral necrosis of the renal cortex is the prominent feature, and results from platelet-fibrin thrombi occluding predominantly the renal circulation [2]. A TMA syndrome can also be observed in association with pregnancy, cancer and chemotherapy, human immunodeficiency (HIV) infection, malignant hypertension, or following hematopoietic stem cell transplantation.

Advances in recent years have delineated the molecular mechanisms of some TMA syndromes, including TTP, some atypical HUS and HELLP syndrome. These studies have clearly revealed that TMA syndromes encompass several distinct molecular defects, although the cause of TMA might not be immediately apparent from the clinical presentation. Concerning nomenclature and syndromic definitions, we use here distinctively the terms TTP and HUS to identify TMA syndromes fulfilling typical clinical and biological presentations of these respective entities. When the underlying cause of disease is uncertain, the term TMA is preferred. This work, based on the experience of the French group, aims to present the most recent physiopathological and therapeutic data in the field of TTP, HUS, and other TMA syndromes.

THE FRENCH REFERENCE CENTRE FOR THE MANAGEMENT OF THROMBOTIC MICROANGIOPATHIES

The French network on Thrombotic Microangiopathies is a multidisciplinary collaborative group involving hematologists, resuscitators, nephrologists, immunopathologists and
Role of Von Willebrand Factor and ADAMTS13 Deficiency

VWF is a multimeric protein involved in the initiation of platelet clumping. It is synthesized and released by endothelial cells and stored in cytoplasmic organelles called Weibel-Palade granules. The basal structure of VWF is a 200 to 300 kDa monomer. Those monomers are linked by disulfide bonds and form multimers of 500 to 20-30000 kDa with a globular conformation which diminishes VWF-platelet interaction. In high shear stress conditions, however, those high molecular weight VWF multimers become unfolded and display many binding sites for ligands, enhancing their heomostatic activity [8].

The link between VWF and TTP pathophysiology was provided by the seminal work of Moake [9], who found that patients with chronic relapsing TTP displayed large amounts of circulating high molecular weight VWF at the acute phase of the disease and during remission. Since these unusually large VWF multimers are absent in normal plasma, Moake hypothesized that a yet undiscovered plasma protease was involved in the cleavage of hyper-adhesive VWF multimers. This protein was purified in 1996 by Tsai and Furlan independently [10] [11], and cloned in 2001 [12]. It is a specific zinc metalloproteinase called ADAMTS13 (acronym for A Disintegrin And Metalloproteinase with ThromboSpondin-1 motifs), that specifically cleaves unfolded, high molecular weight VWF multimers between tyrosine 842 and methionine 843 residues of A2 domain in high shear stress conditions (Fig. 2). As a consequence, a dysfunction of ADAMTS13 leads to a persistent VWF-dependent platelet accumulation, eventually causing microvascular thrombosis and TTP (Fig. 3).

The ADAMTS13 gene contains 29 exons spanning approximately 37 kb on chromosome 9q34. It encodes a 4.7-kb transcript that is expressed primarily in the stellate cells of the liver (Ito cells) and a 2.4-kb transcript in multiple tissues including placenta, skeletal muscle and certain tumor cell lines. Plasma ADAMTS13 concentration in about 1 μg/ml and its evaluated plasmatic half-life is 3 days [13]. Until
now, ADAMTS13 remains the only enzyme involved in the cleavage of VWF multimers. However, it is not excluded that other proteins may act in concert with ADAMTS13 to process VWF multimers. Indeed, thrombospondin-1 (TSP-1) is an enzyme with reductase activity towards VWF disulfide bonds, which may facilitate ADAMTS13 access to VWF cleavage sites [14]. By regulating VWF multimers size, ADAMTS13 was also reported to have a role in the regulation of leukocytes rolling and adhesion on endothelial cells and in neutrophils extravasation during inflammation [15]. In addition, although not yet observed in humans, complete deficiency of ADAMTS13 in mice was reported to be prothrombotic, even in the absence of findings consistent with TTP [16].

In normal healthy individuals, ADAMTS13 activity ranges between 50 and 150 p. cent. ADAMTS13 activity is often mildly decreased (20 to 40 p. cent of activity) in various contexts such as disseminated intravascular coagulation, liver cirrhosis, severe sepsis, idiopathic thrombocytopenic purpura, or systemic lupus erythematosus (not associated with TMA) [17] [18]. A low ADAMTS13 activity was also reported in particular physiological conditions, such as elderly, neonates, during pregnancy, or after a surgical procedure [19] [18]. The significance of this result is still unclear; it may result from a transient disturbance in protein synthesis, and/or consumption in relation with an increase in circulating VWF multimers concentrations. These results are in sharp contrast to the extremely low levels (less than 5 p. cent of normal values) of ADAMTS13 activity observed in 70 to more than 90 p. cent of patients with TTP [20] [21] [22]. Moreover, ADAMTS13 activity was found normal or at least detectable in most HUS cases [20] [23], or in other TMA syndromes including hematopoietic stem cell transplantation-associated TMA, HELLP syndrome, or catastrophic antiphospholipid syndrome.

Mechanisms of ADAMTS13 Deficiency in TTP

The severely decreased ADAMTS13 activity observed in TTP may result from biallelic mutations of ADAMTS13 gene (5 to 10 p. 100 of cases), which mainly involve pediatric cases, or from autoantibodies directed to ADAMTS13 (90 to 95 p. 100 of cases), corresponding mostly to adult forms [13].

ADAMTS13 Deficiency in Congenital TTP

Congenital TTP, previously known as Upshaw-Schulman syndrome, is an autosomal recessive disease resulting from biallelic mutations of ADAMTS13 gene. Until now, more than 70 candidate mutations were reported, which involve amino-acid substitutions (60 p. cent of cases), non-sense mutations, or frameshift mutations (20 p. cent both). Half mutations occur in the catalytic domain, the cystein-rich domain, and the spacer domain. These mutations have 2 functional and non exclusive consequences. The first is the direct loss of catalytic activity. In vitro experiments showed that the deletion of the carboxy-terminal end of ADAMTS13
gene, including the CUB domains, the TSP-1 motifs and the spacer domain only mildly reduced the enzyme activity, whereas the deletion of the desintegrin-like domain results in a severely decreased altered vWF cleavage. In patients however, mutations were found all over ADAMTS13 gene, suggesting that all domains are critical for an optimal enzymatic activity in vivo [24]. The second consequence of these mutations is a defect in the release of ADAMTS13 by their secreting cells, which may occur at the lateral pole of the cells [13].

So far, no clear correlation could be established between ADAMTS13 mutations and clinical presentation, suggesting the involvement of additional factors in TTP pathophysiology. These may include infectious processes (extrinsic factors), but also yet unknown “modifying genes” (intrinsic factors) which may act in concert with ADAMTS13 in various processes such as platelets aggregation, endothelial activation, or vascular homeostasis. This view was strongly supported by the analysis of ADAMTS13-deficient mice, in which intravenous injection of a microbe-derived toxin derived from bacterial pathogens associated with HUS resulted in a striking syndrome closely resembling human TTP. Importantly, this syndrome only occurred in Casa/Rk strain mice, which harbor 5 to 10 times higher circulating VWF multimers. Moreover, the absence of correlation between circulating VWF multimers concentration and the severity of TTP led the authors to conclude that additional genes may be involved in this process [25]. Interestingly, this relevant model of congenital TTP also allowed demonstrating the crucial role of VWF in TTP since CASA/Rk/Adamts13-/- mice in which VWF gene was also invalidated did not develop TTP after shigatoxin administration [26].

**ADAMTS13 Deficiency in Acquired TTP**

In acquired TTP, ADAMTS13 deficiency usually results from autoantibodies altering the protein activity, leading to consider acquired TTP as an autoimmune disease. The efficiency of immunomodulating agents such as rituximab in the prevention of relapses in patients with persistent anti-ADAMTS13 antibodies further comforted this view. In 38 to 95 p. cent of cases, anti-ADAMTS13 antibodies display an inhibitory activity against the enzyme in vitro, as evidenced by mixing the plasma of a patient with acquired TTP with normal human plasma, thereby neutralizing the enzyme activity of this latter [23] [27]. The wide range of these results probably reflects the lack of standardization and reproducibility of the assays for ADAMTS13 functional assays. In patients with no detectable inhibitory anti-ADAMTS13 antibodies, ADAMTS13 deficiency may be related to inhibitory antibodies which concentration is below the detection threshold of the current assays, or either to non-neutralizing...
antibodies, which are identified by ELISA [28]. In most cases, anti-ADAMTS13 antibodies (either inhibitory or non-inhibitory) are of IgG type. In rare cases, IgA and/or IgM isotypes were associated with antibodies of IgG isotype, and may worsen the prognosis [28] [29]. Anti-ADAMTS13 antibodies may decrease ADAMTS13 activity by directly inhibiting the catalytic activity, or by decreasing protein concentrations through an opsonization process [29]. The inhibition of ADAMTS13 binding to its endothelium receptor CD36 is a tempting possible mechanism that still remains to be demonstrated. Anti-ADAMTS13 may recognize various epitopes among the protein. Antibodies directed against the cysteine-rich and the spacer domains are the most frequently observed. These may be associated with antibodies directed against CUB domains, the first thrombospondin-1 (TSP-1) motif, or the region including the catalytic domain, the desintegrin-like domain, and the first TSP-1 domain. More rarely, these antibodies recognize the TSP-1 motifs 2 to 8, or the propeptide domain [30]. Anti-ADAMTS13 IgG, as detected by ELISA, were found in a majority of patients with severe ADAMTS13 deficiency (35/36 patients, 97 p. 100), but also in 3 patients with TTP showing a detectable ADAMTS13 activity (11 to 16 p. 100 of normal activity), which suggests that ELISA sensitivity may be higher than that of functional ADAMTS13 assays [31]. On the opposite, these antibodies were not found in 4 patients with a diagnosis of HUS [31]. Those promising results, suggesting a specificity of anti-ADAMTS13 for acquired TTP, need however confirmation on larger series of patients.

**Clinical Presentation**

TTP is a specific subset of TMA usually characterized by a microangiopathic hemolytic anemia with a profound peripheral thrombocytopenia, fever, central nervous system manifestations, and renal failure [5]. However, these features are complete only in 40 p. cent of cases; in other instances, TTP may be revealed by only a bicytopenia without organ involvement. Indeed, the only association of a microangiopathic hemolytic anemia with a peripheral thrombocytopenia should alert clinicians for this diagnosis. Importantly, reports clearly emphasized that TTP may be underestimated and under-recognized, particularly in children. As a general rule, TTP should be systematically suspected in children with a diagnosis of peripheral cytopenia, particularly if associated with any organ involvement. In patients with an apparent diagnosis of idiopathic thrombocytopenic purpura or Evan’s syndrome not responding to usual therapies, the identification of schistocytes on repeated blood smears, in association with a severe ADAMTS13 deficiency may allow revisiting the diagnosis [32].

Central nervous system involvement occurs in 84 to 92 p. cent of cases and was shown to be associated with a worse prognosis by some groups [33]. It may result in confusion, obtundation, headache, somnolence, coma and seizure. A focal sensitive or motor deficiency, dysarthria, or aphasia may also be observed. Brain magnetic resonance imagery may disclose punctiform images in white matter resulting from ischemia. Thrombosis or hemorrhage may also be observed. Fever is observed in 59 to 98 p. cent of cases. Renal failure is usually mild and is observed in half of cases. A mild proteinuria and/or hematuria may also be observed. Heart involvement may be characterized by thoracic pain, repolarization or conduction changes, and elevated troponin Ic, which may reflect left ventricular dysfunction [34]. Digestive involvement, associating vomiting and abdominal pain with sometimes pancreatitis, or more rarely, lung and ocular injury, may occur.

Histopathologically, TTP is characterized by the presence of widespread hyaline thrombi in the terminal arterioles and capillaries, accounting for organ failure. The high levels of shear stress in the arterioles and capillaries are believed to be a critical factor in determining the distribution of thrombosis in TTP. Autopsic studies revealed that thrombi involved most organs, including the brain (mainly cerebral cortex), heart, spleen, pancreas, adrenal gland and kidney. Thrombi are composed primarily of platelets and VWF, which contrasts with the thrombi of disseminated intravascular coagulopathy or the HUS, which are rather characterized by prominent fibrin deposits [35].

**Acquired TTP**

Acquired TTP occurs more frequently in females (3 females for 2 males), within the fourth decade. The onset of disease is typically sudden. However, prodromic manifestations including fatigue, arthralgias, myalgias, abdominal or lumbar pain, suggesting a flu-like episode are frequently observed. The incidence of TTP was reported to be of 4 cases per million hab. per year. Acquired TTP could be associated with various autoimmune diseases, especially SLE (4 p. 100 of SLE cases) [36]. More generally, acquired TTP is associated in one third of cases with various manifestations suggesting an underlying autoimmune process. Antinuclear antibodies or antibodies directed against CD36 are observed in up to two third of cases [37] [38]. Finally, questioning the patients with an acquired TTP allow sometimes evidencing a history of autoimmune disease in other family members. These observations, arising from series of adult patients or either from more limited series of pediatric cases [39], highly suggest that patients with acquired TTP have a trend to develop autoimmune manifestations. In addition, multiple studies focused on the higher prevalence of acquired TTP in particular ethnic groups, such as Afro-Caribbeans [27] [38] [40], suggesting the existence of susceptibility genes involved in the loss of tolerance of the immune system towards ADAMTS13. The report of acquired TTP in two twin sisters at 23 and 24-year old further supports this hypothesis [41].

Whether anti-ADAMTS13 antibodies have a prognostic value in TTP is the matter of intensive research. The identification of inhibitory anti-ADAMTS13 antibodies was associated with a longer time to platelet count recovery and larger volumes of plasma, in relation with more frequent early relapses during intensive treatment or during the decrease of plasma exchange sessions [42] [43] [44]. In addition, a high titre of anti-ADAMTS13 antibodies (either inhibitory or non-
to 40 p. cent of cases, patients experience one or multiple plasma exchanges sessions remains a matter of debate. In 30
platelet recovery. The rapid or progressive decrease in elusiveness. Plasma exchanges are performed daily until durable
importance of these latter in patients’ improvement remains elusive. Plasma exchanges are performed daily until durable and non-neutralizing anti-ADAMTS13 antibodies and drawing definitive conclusions.

**Congenital TTP**

Congenital TTP, previously termed Upshaw-Shulman syndrome, is an autosomal recessive disorder usually observed in children and newborns. Consequently, probands may be either male or female and parents (who may be consanguineous) are healthy with a mild decrease in ADAMTS13 activity. Congenital TTP may be revealed by cytopenias or jaundice in the neonatal period (about 75 p. cent of cases) or later, usually in the first decade [24]. Some congenital TTP may be revealed in adulthood [47], particularly within a context of pregnancy. Clinical manifestations typically include anemia, thrombocytopenia, and organ involvement of variable severity. In neonates, unexplained hemolysis and thrombocytopenia sometimes result in exsanguino-trans-fusion sessions. Patients frequently experience repeated relapses at varying intervals that are frequently triggered by non-specific infectious processes, including flu-like episodes. Initially, TTP episodes may resolve completely; however, when multiple relapses occur, those patients with a chronic relapsing form may develop a chronic renal failure or ischemic lesions in brain. In other instances, patients may present with moderate chronic hemolysis and thrombocytopenia. A history of hemolysis or thrombocytopenia may be found in other family members.

**Treatment and Outcome**

**Plasmatherapy**

Without treatment, acquired TTP is almost always fatal, whereas under rigorous management, up to 80 p. cent of patients recover. Treatment is based on therapeutic plasma exchanges [48] [49], which have to be started as soon as the diagnosis is established or even suspected. If plasma exchanges cannot be performed immediately, high dose plasma infusions should be started in emergency until plasma exchange availability [50]. Plasma exchanges allow bringing large volumes of plasma that will supply ADAMTS13 deficiency. Plasma exchanges have additional therapeutic effects such as removal of anti-ADAMTS13 antibodies or of high molecular weight VWF from plasma; however, the importance of these latter in patients’ improvement remains elusive. Plasma exchanges are performed daily until durable platelet recovery. The rapid or progressive decrease in plasma exchanges sessions remains a matter of debate. In 30 to 40 p. cent of cases, patients experience one or multiple relapses after the initial episode, which require to resume intensive plasmatherapy. The period between relapses may range from days to many years. In 10 p. cent of cases, TTP is unresponsive to plasma exchanges and requires more intensive plasmatherapy and/or immunomodulatory therapies.

Patients with congenital TTP are efficiently treated with plasma infusions. In patients with chronic relapsing forms or persistent hemolysis and thrombocytopenia, prophylactic infusions every 2 to 3 weeks may efficiently control the disease and prevent serious complications. Other patients may maintain normal or mildly subnormal platelet counts and require plasma infusion only during intermittent acute exacerbations.

**Immunomodulatory Therapies**

The view that acquired TTP is an autoimmune disease incites the use of immunomodulatory therapies. Steroids are frequently proposed at the acute phase of the disease, in association with plasma exchanges, but their efficacy was not evaluated on controlled studies. Other immunomodulatory agents such as cyclosporin A, azathioprine, or vincristine were also used efficiently in single cases or small series of patients. Patients with a refractory, threatening disease may be efficiently treated with pulses of cyclophosphamide [51].

These last years, the chimeric monoclonal antibody rituximab, directed against CD20 antigen on B lymphocytes, became the most frequently used immunomodulatory agent. Indeed, patients with a refractory disease or showing intermittent relapses were reported to be efficiently treated with high response rates, along with an increase in ADAMTS13 activity and a decrease in anti-ADAMTS13 antibodies [52] [53]. However, the use of rituximab may not systematically hamper long term relapses. Ongoing large, prospective studies are attempting to specify the schedule of rituximab administration in order to reduce the number of plasma exchanges, which are associated with significant side-effects.

**HEMOLYTIC UREMIC SYNDROME**

Two forms of HUS are classically identified. The first occurs after an intestinal infection resulting from a shiga-toxin-producing Escherichia coli (STEC) strain; it is therefore termed STEC-associated HUS or diarrhea-associated HUS (D+ HUS), and represents the most common form of HUS (for an extensive review, see [54]). The second form, termed atypical HUS, occurs out of a context of STEC infection and accounts for 5 to 10 p. cent of all cases of the disorder. It was associated with congenital or, more rarely, acquired complement pathway dysfunctions [55]. TMA with features of atypical HUS were also reported in patients with abnormalities in cobalamins metabolism, or either in association with various conditions such as HIV infection, autoimmune diseases, cancers and pregnancy. These latter are thought to act as triggers causing endothelial cell activation and injury, probably within a context of genetic susceptibility [55].
Pathophysiology

**STEC-Associated HUS**

Toxins released by shigatoxin-producing microorganisms, most commonly Escherichia coli O157:H7 (shigatoxins 1 and 2) and Shigella dysenteriae serotype 1 (shigatoxin) strains, are directly involved in D+ HUS pathophysiology since their injection to non human primate leads to manifestations mimicking HUS in a dose-dependent manner [56]. Shigatoxin is composed of one A subunit of 33 kDa and 5 B subunits of 7.7 kDa each. Most E. coli O157:H7 carry the gene encoding shigatoxin 2, and about two-thirds have the gene encoding shigatoxin 1. Other strains have also been involved: E. coli O111: H-, O26: H11 and O103: H2. Bacterial agents ingested from contaminating sources proliferate in the intestinal lumen and adhere to mucosal epithelial cells of the colon. Shigatoxins damage the underlying tissue and vasculature and cause bloody diarrhea. The lesions are potentiated by neutrophils, which are recruited into the damaged colon and activated by IL-8 and other chemokines. Shigatoxins cross the intestinal barrier and are transported by neutrophils, monocytes and platelets in blood flow onto renal microcirculation. The toxins stimulate the release of TNF, IL-1 and IL-6 from monocytes and renal glomerular and tubular epithelial cells, which up-regulate the expression of shigatoxins receptors called globotriaosylceramide and galabiosylceramide.

Shigatoxins bind to their receptors through B subunits on glomerular capillary endothelial cells, mesangial cells, and glomerular and tubular epithelial cells. After internalization, subunit A of shigatoxins inhibits 28S ribosomal subunit, thereby inhibiting protein synthesis machinery. This process leads to endothelial cell apoptosis and endothelial injury. This damage is potentiated by the monocytes and neutrophils that infiltrate the glomeruli in response to the secreted chemokines such as IL-8 and fractalkine and the production of monocyte chemoattractant protein 1 by renal cells. Renal endothelial cell injury results in the expression of high molecular weight VWF multimers and adhesion molecules, such as P-selectin, PECAM-1 (platelet-endothelial-cell adhesion molecule 1) and vitronectin (αβ3 integrin) receptors. Damaged endothelial cells also express high levels of tissue factor, plasma activator inhibitor type 1 (PAI-1) and D-dimers. All these features lead to a local prothrombotic state resulting in increased platelet adhesiveness with fibrin-rich microthrombi (Fig. 4).

It is estimated that only 5 to 15 p. cent of patients with STEC-associated gastro-enteritis develop a full-blown HUS, suggesting the involvement of yet unknown additional factors. These may include mutations or polymorphisms in putative susceptibility genes including shigatoxin receptor, TNFα and IL-1, as well as their receptors.

**Atypical HUS**

In children, as well as in adults, atypical HUS was associated in up to 50 p. cent of cases with genetic changes in genes involved in key regulators of alternative complement pathway. Familial occurrence of atypical HUS has been recognized for many years. Inheritance is now thought to be dominant with a global 50 p. cent penetrance. In 1998, Warwicker et al. could show a segregation of the disease to the q32 region of chromosome 1 [57], which contains genes involved in the regulation of complement activation.

The complement system is an ancient innate immune network of plasma proteins that began evolutionary as a host defense system of hemolymph. The oldest cascade, the alternative pathway, allows to rapidly coat invading microbes with large quantities of the opsonic complement fragment...
C3b (Fig. 5). This process is facilitated by an amplification loop that results in the deposition of several million molecules of C3b on bacteria within a few seconds. Complement activation may also occur on altered self-tissues, such as on cells undergoing apoptosis and at sites of injury and infection. To prevent excessive production and deposits of C3b, the alternative pathway is finely regulated by proteins that prevent C3 activation. Indeed, heterozygous mutations (haploinsufficiency) in these regulators predisposes humans to HUS [58].

**Complement Proteins Implicated in Atypical HUS**

The first complement protein associated with atypical HUS was factor H, a 150-kDa plasma protein composed of 20 short consensus repeats of 60 amino-acids each. Factor H normally protects host cells from accidental damage by the alternative complement pathway by displacing Bb from C3b, thereby exposing C3b to cleavage and inactivation by factor I. Mutations in the factor H gene in atypical HUS have now been widely described. More than 100 disease-associated mutations are reported in the factor H-HUS mutations database (http://www.FH-HUS.org). Twenty to 30 p. cent of patients with atypical HUS were reported to harbour heterozygous mutations in factor H [59]. Missense mutations, deletions and frame shifts in the factor H gene have been identified in patients and their relatives. Most mutations cluster in short consensus repeat number 20 of factor H gene in the C-terminal end of the protein, which disrupts a heparine-binding site involved in factor H binding to host surfaces. In most cases, they consist in missense mutations and are associated with normal levels of circulating factor H. In the remaining cases, they result in either a truncated protein or impaired secretion of the protein and thus cause a 50 p. cent reduction in plasma levels of factor H. A mouse model of HUS designed to mirror human mutations in factor H confirmed that the binding of factor H in anionic targets such as heparine on endothelial cells surface had a crucial importance since suppression of this function resulted in the occurrence of HUS features [60]. Of note, mutations were also observed in genes harbouring sequence homologies with factor H gene (CFHR [complement factor H-related]1 and CFHR3), as well as fusion genes between factor H and CFHR1. An acquired dysfunction in factor H related to anti-factor H antibodies of IgG type was also reported in 3 pediatric cases of atypical HUS [61]. Those antibodies are usually associated with mutations of CFHR1.

Further proteins involved in complement regulation, such as factor I or CD46/MCP (membrane cofactor protein) were also found mutated in atypical HUS [62] [63] [64] [65]. Mutations in factor I were reported in < 4 p. cent to 13 p. cent of cases. In 30 p. cent of cases, this latter is associated with additional mutations of complement components or with anti-factor H antibodies. Mutations in CD46/MCP were reported in 13 p. cent of cases [59]. Mutations in the gene encoding factor B were found to enhance formation of the alternative C3 convertase C3bBb or increase resistance to inactivation [66]. More recently, heterozygous mutations in complement C3 were identified in 11 patients and 3 relatives with atypical HUS. These were heterozygous missense or nonsense mutations, which resulted in most cases in a gain of function of complement activation through a reduced interaction with MCP or factor H (the major inhibitors of the alternative complement pathway) [67].

The specific role of these abnormalities in the occurrence of HUS features still remains hypothetical. They could result

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**Fig. (5). Key regulators of alternative complement pathway involved in atypical HUS.** Mutations in factor H, factor I, CD46/MCP (membrane cofactor protein), as well as auto-activating mutations in complement factor B and C3 were reported. Factor H dysfunction may also involve autoantibodies. It is hypothesized that those mutations result in excessive complement activation, with renal endothelial cells damage and hyperexpression of tissue factor, fibrin formation and thrombi formation. : mutated protein.
in an excessive activation of complement pathway mediated by infectious agents or immune complexes, with a subsequent release of excessive amounts of complement components such as C5 and membrane attack complex. As a consequence, damaged renal endothelial cells may express procoagulant proteins including local tissue factor with factor VIII binding and activation, leading to the generation of thrombin and fibrin polymers (Fig. 5). Noteworthy, results obtained from patients and analysis of animal models clearly emphasize that atypical HUS appears more and more as a multigenic disease involving multiple complement genes in an individual patient. Therefore, those mutations must be considered to be predisposing rather than causative, as a trigger factor (such as infections or a pregnancy) is necessary to initiate the disease [58].

Interestingly, factor H dysfunctions were also reported in other diseases in which this protein may have a role in protecting cells against various insults. Indeed, age-related macular degeneration was associated with a polymorphism in short consensus repeat 7 of factor H [68], whereas membrane- proliferative glomerulopathies were associated with a complete deficiency in factor H. Mutations in factor H, but also in factor I and CD46/MCP were reported in HELLP syndrome [69], suggesting that this disorder may be an additional form of TMA with complement dysfunction as risk factors.

**Streptococcus Pneumoniae-Associated HUS**

HUS associated with a pneumococcal infection is a specific form of HUS, resulting from the expression of Thomsen-Friedenreich antigen at the surface of erythrocytes, endothelial cells, and glomerules. This antigen, which is normally covered by sialic acid, is exposed by the pneumococcal-secreted neuraminidase, and subsequently recognized by circulating IgM, leading to platelet aggregation with endothelial and glomerular lesions. Consequently, plasmatherapy is usually contra-indicated in this form of HUS since IgM provided by plasma infusions may exacerbate the disease.

**HUS Associated with Cobalamin Disorders**

Atypical HUS was associated with dysfunctions of cobalamin metabolism disorders. Cobalamin (vitamin B12) derivatives, methylcobalamin and adenosylcobalamin, participate as cofactors for the enzymes 5-methyltetrahydrofolate-homocysteine methyltransferase and methylmalonyl-CoA mutase. These enzymes are involved in the remethylation of homocysteine to methionine and in the conversion of L-methylmalonyl-CoA to succinate, respectively. Complementation studies subgroup Cbl disorders from cblA to cblH. CblC complementation group is the principal inborn error of Cbl metabolism associated with atypical HUS. Renal complications of CblC disease are thought to be mainly due to hyperhomocysteinemia-induced damage to glomerular endothelium [70].

**Clinical Presentation**

D+ HUS represents more than 90 p. cent of HUS cases in children before 3 year-old; therefore, any child presenting for the first time with a TMA syndrome, but who does not have diarrhea, could still be infected with an STEC. Those patients should thus be investigated for the presence of an STEC by microbiological analysis of the stools and urines. The incidence of diagnosed E. coli O157:H7 infections is greater among rural than urban populations, probably because of greater exposure to animals excreta. Transmission from cattle to people might be airborne. D+ HUS may occur within an epidemic context, mostly in the summer and autumn. In Buenos-Aires (Argentina) where STEC infections are endemic, HUS has a very high incidence. Bacterial strains can contaminate unpasteurized milk or dairy products, ground beef, insufficiently cooked food, as well as municipal or swimming water. Diarrhea occurs 2 to 12 days later, and becomes bloody after 1 to 3 days. Abdominal pain may be intense and greater than is generally observed in other forms of bacterial gastroenteritis. Defecation may also be painful.

Cerebral manifestations are important determinants of morbidity and mortality. They can be observed in 50 p. cent of cases. Analysis of blood pressure and metabolic parameters on admission did not predict which child would exhibit cerebral signs. During the course of the illness however, children with cerebral involvement had more severe azotemia, lower minimum sodium concentrations and required more dialysis [71]. Cerebral magnetic resonance imaging (MRI) and computerized tomography may evidence features of cerebral oedema within parieto-occipital white matter, suggestive of posterior leuko-encephalopathy. These features were more frequently observed in patients with severe high blood pressure. Patients with seizure and/or coma may display ischemic lesions of basal ganglia with hemorrhagic infarction [72].

Other non-renal complications such as cardiac dysfunction, intestinal complications including perforation and necrosis, and pancreatitis have been reported more rarely.

**Treatment and Outcome**

HUS management systematically requires a symptomatic treatment, including haemodialysis, control of renin-dependent high blood pressure with angiotensin convertase inhibitors or angiotensin receptors antagonists. In D+ HUS, intravenous rehydration with isotonic crystalloid and maintenance fluid provide optimum nephroprotection. Plasmatherapy does not seem to modify the prognosis, which remains good under symptomatic treatment. Antibiotics, anti motility agents or narcotics were associated with a worsening of the disease, and should thus be avoided. Non-steroidal antiinflammatory agents, by diminishing renal blood flow, should not be used either. Prognosis of D+ HUS is usually good, and end stage renal failure or death are observed in up to 12 p. cent of cases, with 25 p. cent of survivors demonstrating long term renal sequelae [73].

Atypical HUS is typically characterized by a high mortality rate (54 p. cent). About half of survivors experience relapses, and over one third require long-term dialysis. From pediatric series, children with factor H or factor I mutations...
develop relapses leading rapidly to end-stage renal failure and/or death. Factor H mutations were associated with the most severe prognosis, since 60% of patients reached end stage renal failure or died within <1 year. Half of patients with factor I mutation have a rapid evolution to end stage renal failure, and half recover. Patients with factor H or factor I mutations usually relapse after renal transplantation. Patients with CD46/MCP mutations have a better prognosis characterized by a relapsing course without end stage renal failure. Moreover, the rate of relapse after kidney transplantation is low in this group [74].

Plasma exchanges were reported to have a beneficial effect in one third of children from all groups except for patients harbouring MCP mutations [74]. In atypical HUS with factor H mutations, kidney and liver transplantations were intended, but this procedure is associated with a significant mortality risk [75]. Targeted therapies aimed at supplying the abnormal proteins or inhibiting complement pathway should be evaluated in the forthcoming years.

HUS associated with cobalamin disorders require parenteral administration of hydroxocobalamin [70].

OTHER TMA SYNDROMES

Pregnancy and Post-Partum

TMA occurring in the setting of pregnancy and post-partum may display features of acquired or congenital TTP, as well as HUS or HELLP syndrome. Indeed, pregnancy may reveal ADAMTS13 or complement dysfunctions resulting from gene mutations [69]. HELLP syndrome needs to be identified since it implies a foetal extraction, whereas TTP may be efficiently treated with only plasma exchanges. Liver involvement disseminated intravascular coagulation and detectable ADAMTS13 activity, usually higher than 20 p. cent, which are typical features in HELLP syndrome but not in TTP, may help to distinguish both diseases. Severe eclampsia and HELLP syndrome may result from a maternal endothelium dysfunction mediated by an excessive release of both placenta-derived soluble VEGF (vascular-endothelium growth factor) receptor and endoglin. This latter is a circulating transforming growth factor (TGF)-β1 co-receptor which impairs binding of TGF-β1 to its receptor. In concert with soluble VEGF receptor, endoglin may inhibit downstream signalling including effects on activation of eNOS (endothelial nitric oxide synthetase) and vasodilatation, and thereby induce placental hypoperfusion.

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT)-associated TMA was initially considered as a particular form of TTP with a worse prognosis due to treatment refractoriness. ADAMTS13 activity was found consistently normal in this form of TMA. Consequently, this latter now tends to be individualized as a specific form of TMA with specific pathophysiology and prognosis. HSCT-associated TMA is favoured by numerous initiating factors, which include total body irradiation in transplant conditioning, infections, medications such as calcineurin inhibitors, as well as graft versus host disease grade 2 to 4 [76]. The response to plasma exchange is disappointing, and the management should include as much as possible the treatment of triggering factors. Various studies have reported the efficiency of defibrotide, which is a single strand polyribonucleotide obtained from mammal DNA. This latter may protect endothelial cells from TNF-α-associated cell death.

Cancers

Stomach, breast and prostate cancers are the most typical malignancies providing a TMA syndrome. Cancer-associated TMA onset is usually insidious. Dyspnea, wasting, severe disseminated intravascular coagulopathy with dacryocytes and massive erythrocytoses are specific features [77]. Bone marrow investigations frequently display extrahematopoietic, metastatic cells. TMA pathophysiology still remains unclear. Metastatic micro-emboli may be involved in the occlusion of microvessels, thereby inducing erythrocytes fragmentation and platelet activation. Cytokines such as TNFα may also participate to endothelial aggression. In this context, ADAMTS13 activity is usually measurable and higher than 20 p. cent. Rare cases of severe acquired ADAMTS13 deficiency were observed; these latter may correspond to a paraneoplastic form of TTP (Oberic et al., submitted). Diagnosis of cancer-associated TMA is very poor, and depends on treatment responsiveness of the underlying malignancy.

Medications

A large number of drugs were associated with TMA, including antiplatelet agents, antineoplastic drugs and quinine.

TTP with a documented antibody-mediated severe ADAMTS13 deficiency was reported in a small fraction of patients treated with ticlopidine, an inhibitor of one of the platelet adenosine diphosphate receptors, and clopidogrel, the structurally similar agent [78] [79]. The estimated incidence of ticlopidin-associated TTP is 1 per 1600 to 5000 patients treated, with a disease onset that ranged from 2 to 12 weeks following treatment initiation [80]. Though no clopidogrel-associated cases were initially observed among 20,000 closely monitored patients treated in phase 3 clinical trials and cohort studies, patients were found to develop acquired TTP within the first two weeks following drug intake, suggesting a possible causal relationship. The mechanism leading to anti-ADAMTS13 antibodies production remains unknown. It is not excluded however that ticlopidin may induce the development of anti-ADAMTS13 autoimmune reaction against ADAMTS13 in a mode analogous to the development of anti-red cell antibodies in association with the use of alpha-methyldopa. Additionally, ticlopidin was reported to disrupt production of extracellular matrix components critical for microvascular endothelial cell integrity with induction of apoptosis [81]. Treatment with plasma exchange usually allows obtaining resolution of TTP in 77 to 84 p. cent of cases [80]. Relapses may occur in clopidogrel-associated TTP [79].
Table 1. To a molecular-based classification of TMA syndromes

<table>
<thead>
<tr>
<th>Severe ADAMTS13 deficiency (TTP)*:</th>
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<tbody>
<tr>
<td>1. TTP + ADAMTS13 mutations</td>
</tr>
<tr>
<td>2. Autoimmune TTP:</td>
</tr>
<tr>
<td>+ HIV</td>
</tr>
<tr>
<td>+ Antiplatelet agents</td>
</tr>
<tr>
<td>+ Cancers</td>
</tr>
<tr>
<td>+ Pregnancy</td>
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<tr>
<td>“Idiopathic”</td>
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</table>

<table>
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<tr>
<th>Detectable ADAMTS13 activity (HUS):</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HUS + enteropathogenic bacteria</td>
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<tr>
<td>2. Atypical HUS + complement dysfunction</td>
</tr>
<tr>
<td>Mutations: factor H, CFHR, FI, MCP/CD46, factor B, C3</td>
</tr>
<tr>
<td>Auto-Abs: Anti-factor H antibodies</td>
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<tr>
<td>3. Atypical HUS + S. pneumoniae</td>
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<tr>
<td>4. Atypical HUS + Cobalamin metabolism disorders</td>
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<table>
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<tr>
<th>Detectable ADAMTS13 activity (others):</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Other TMA syndromes:</td>
</tr>
<tr>
<td>+ HIV (often AIDS)</td>
</tr>
<tr>
<td>+ Connective tissue disease</td>
</tr>
<tr>
<td>+ Medications</td>
</tr>
<tr>
<td>« Idiopathic »</td>
</tr>
<tr>
<td>2. HELLP Syndrome</td>
</tr>
<tr>
<td>+ sVEGF-R1, sEndoglin and/or complement dysfunction</td>
</tr>
<tr>
<td>3. Malignant AHT, CAPS, Type II HIT, VOD, severe DIC</td>
</tr>
</tbody>
</table>

* includes rare cases of TTP with decreased but detectable (10-20%) ADAMTS13 activity and anti-ADAMTS13 antibodies [31]. CFHR : complement factor H-related genes ; HIV: human immunodeficiency virus ; AIDS: acquired immunodeficiency syndrome. HELLP: hemolysis, elevated liver enzymes, low platelet count. AHT: arterial hypertension. sVEGF: soluble form of VEGF (vascular-endothelium growth factor); sEndoglin: soluble endoglin; CAPS: catastrophic antiphospholipid syndrome ; HIT: heparine-induced thrombocytopenia; VOD: veno-occlusive disease; DIC: disseminated intravascular coagulation.

Multiple antineoplastic drugs were associated with TMA. Particularly, mitomycin C induces TMA with a frequency that ranges from less than 2 p. cent of cases to 10 p. cent, and may be dose-dependent. The specific pathophysiological mechanisms may involve a drug-mediated endothelial injury. Mitomycin-associated TMA typically associates high blood pressure with lung oedema. By contrast, central nervous involvement and fever are rarely observed. Response to plasma exchanges is usually poor. More recently, inhibitors of VEGF, aimed at inhibiting tumor vascularization, were associated with nephritic-range proteinuria, high blood pressure and TMA [82], suggesting a major role for VEGF in vascular homeostasis.

HIV Infection

TMA is more frequently observed in HIV-infected patients than in non-infected individuals [83]. However, HIV-associated TMA incidence decreased from 1.4 to 0.3 p. cent since the era of antiretroviral therapies [84] [85]. When compared to HIV-positive patients without TMA, patients with HIV-associated TMA have a lower CD4+ T cell count, a higher HIV RNA viral load and a higher frequency of opportunistic infections. TMA may result from distinct pathophysiological mechanisms. HIV-associated TMA with severe ADAMTS13 deficiency usually have less AIDS-related complications and their median CD4+ T cell count is higher than in patients with a detectable ADAMTS13 activity. In the former, prognosis is comparable to this of non-HIV patients, whereas in the latter, prognosis is poor despite adapted treatment [86-89].

CONCLUDING REMARKS

These last years, our knowledge in TMA pathophysiology outstandingly improved. Indeed, the evidence of a deficiency in ADAMTS13 in TTP allowed understanding the efficiency of plasmatherapy, which opens perspectives of targeted therapies based on recombinant or plasma-purified ADAMTS13. In addition, to consider acquired idiopathic TTP as an autoimmune disease incites to introduce immunomodulatory drugs aimed at reducing the number of plasma exchange sessions. Atypical HUS may also be treated efficiently with purified proteins or complement pathway modulators. Among these latter, eculizumab is a monoclonal antibody directed against C5 component of complement, that was used efficiently in paroxystical nocturnal hemoglobinuria, where a deficiency in GPI-anchored proteins lead to an excessive, complement-mediated, intravascular hemolysis [90].

Altogether, these findings should now allow drawing a rigorous, pathophysiologically-based, TMA classification (Table 1), and defining more homogeneous groups of patients. Obviously, these findings open fascinating perspectives of targeted therapies in the forthcoming years.

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