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REVIEW

Novel developments in thrombotic microangiopathies: is there a common link between hemolytic uremic syndrome and thrombotic thrombocytic purpura?

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Abstract Thrombotic microangiopathies (TMA) represent a spectrum of related disorders associated with newly formed thrombi that block perfusion and thus affect the function of either renal or neurological organs and tissue. Recent years have seen a dramatic development in the field of TMA and for the two major forms hemolytic uremic syndrome (HUS) and thrombocytopenic purpura (TTP), new genetic causes and also autoimmune forms have been identified. This development indicates a similar pathophysiology and suggests that the two acute disorders are based on common principles. HUS is primarily a kidney disease and TTP also develops in the kidney and at neurological sites. In HUS thrombi formation is likely due to a deregulated complement activation and inappropriate platelet activity. In TTP thrombi formation occurs because of inappropriate processing of released multimers of von Willebrand Factor (vWF). Defining both the similarities and the unique features of each disorder will open up new ways and concepts that are relevant for diagnosis, for therapy, and for the prognostic outcome of kidney transplantations. Here we summarize the most relevant topics and timely issues that were presented and discussed at the 4th International Workshop on Thrombotic Microangiopathies held in Weimar in October 2009 (www.hus-ttp.de).

Keywords Thrombotic microangiopathies · Complement · Hemolytic uremic syndrome

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Abbreviations

ADAMTS13	A disintegrin and metalloprotease with thrombospondin motifs
aHUS	Atypical form of HUS
AMD	Age-related macular degeneration
CFHR1	Complement factor H-related protein 1
CRP	C reactive protein
D+−HUS	Diarrhea-associated form of HUS
DEAP-HUS	(Deficient for CFHR proteins and autoantibodies positive for the complement inhibitor factor H)
HUS	Hemolytic uremic syndrome
PNH	Paroxysmal nocturnal hemoglobinuria
SLE	Systemic lupus erythematosus
TMA	Thrombotic microangiopathies
TTP	Thrombotic thrombocytopenic purpura ()
vWF	Von Willebrand factor

Introduction

Thrombotic microangiopathies (TMA) represent a spectrum of related disorders with thrombi formation in the micro-

capillaries that are caused by genetic as well as by acquired factors in the form of autoantibodies. The major two subtypes that present as hemolytic uremic syndrome (HUS) and thrombotic thrombocytic purpura (TTP) are caused by mutations of different genes and also by autoantibodies that target distinct plasma proteins (Fig. 1) [1]. Therefore, it remains a challenge to elucidate the unique features of each disorder as well as the common pathophysiological principles [2].

Hemolytic uremic syndrome

Hemolytic uremic syndrome (HUS) is a disease that is associated with thrombocytopenia, microangiopathic hemolytic anemia, and with acute renal failure. This disease, which was initially described by Gasser and Steck [3] is currently divided into three major sub-forms: typical HUS, atypical HUS (aHUS), and the autoimmune form DEAP-HUS (Fig. 1) [1, 4].

Typical hemolytic uremic syndrome

The typical form, also termed classical HUS, is frequent in children and is often associated with diarrhea and is therefore also termed D+HUS. Typical HUS is in most cases associated with bacterial infections, in particular Shigatoxin-producing enterohemorrhagic bacteria, with the most frequent pathogen being *E. coli* 0175:H7 [4]. However, additional infectious agents like *Shigellae dysententericae* and *Streptococcus pneumoniae* are also associated with typical HUS [5]. Enterohemorrhagic *E. coli* (EHEC) release Shigatoxin or Shiga-like toxins that are linked to pathology and similarly pneumococci secrete neuraminidase [5, 6].

Typical routes of EHEC infections are contaminated food, such as uncooked meat and vegetables as well as unpasteurized milk. Additional infections are mediated by direct person to person contact as it occurs, for example, in nursing homes. Upon treatment classical HUS has a relatively good prognosis. Over the years HUS therapy in

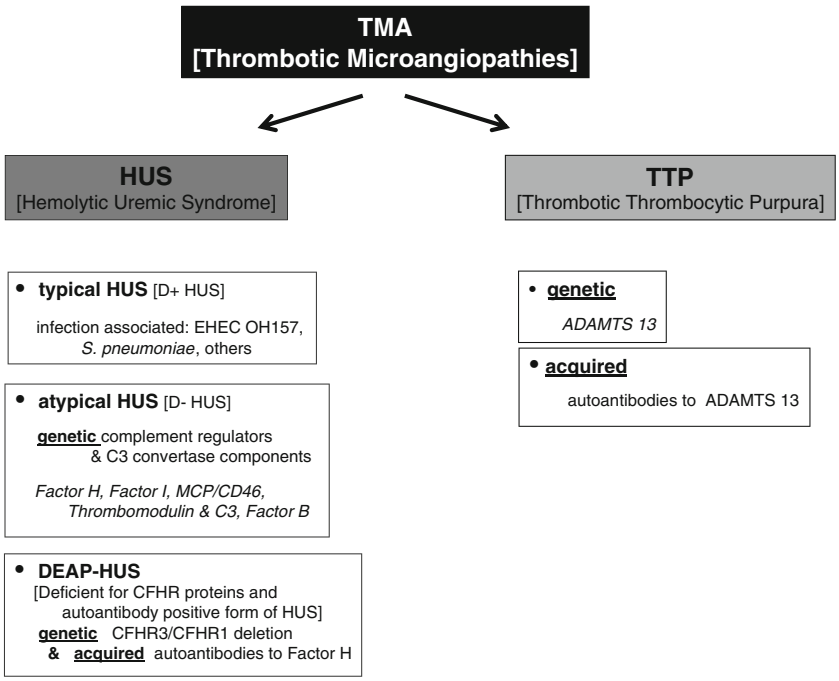

Fig. 1 Different forms of thrombotic microangiopathies. Thrombotic microangiopathies represent a spectrum of related disorders including thrombocytopenia, microangiopathy, and acute renal failure. The general features are subdivided into the two major forms HUS (hemolytic uremic syndrome), where thrombus formation occurs primarily in the kidney, and TTP (thrombotic thrombocytic purpura). Both sub-forms are caused by genetic and also by acquired factors in the form of autoantibodies. In particular for the typical form of HUS that represents approximately 80% of the cases, infections are a particularly inducing trigger. Infections with enterohemorrhagic *E. coli* are frequent and also other pathogens including *Bordetella*, *S. pneumonia*, and *Varicella* infections are reported. However, infections relating to the other two HUS forms are frequently reported. The atypical form of HUS has a genetic cause and seems more frequent in adults. Genes associated with this form of HUS are Factor H, Factor I, MCP/CD46, thrombomodulin, as well as C3 and Factor B, the two components that form the alternative complement pathway C3 convertase. An additional autoimmune form termed DEAP-HUS (deficient for CFHR genes and positive for autoantibodies to Factor H) was characterized to be associated with a genetic factor. Similarly, more recently, TTP that manifests in the kidney and in neurological tissue has a genetic form, in which gene mutations spread along the *ADAMTS13* gene. *ADAMTS13* is a metalloprotease that cleaves the ultra-large multimers of the von Willebrand factor. For the acquired form autoantibodies are identified that bind to the metalloprotease *ADAMTS13*

the form of plasma treatment as well as the better care in intensive care units has reduced the mortality from 8.0 to 0.5% and thus significantly improved the situation of the patients [7, 8]. Despite this progress even typical HUS is still a severe disease and epidemiological follow-up studies show a prevalence of renal damage; however, with an overall good prognosis. Novel genetic data also report variations in complement genes in patients with the typical form of HUS.

Severe defects 1 year after the initial insult are reported in about one third of the patients in the form of arterial hypertension, proteinuria, and a reduced glomerular filtration rate. Upon oral infection enterohemorrhagic *E. coli* (EHEC) binds to the mucosa of the gut. Shigatoxin induces chemokine expression, which results in transmigration of neutrophils from the circulation into the gut. In the blood the toxins bind to neutrophils and are then transported to these target organ, the kidney. Shigatoxin has a modular structure, one subunit of 33 kDa and five beta subunits, each of approximately 7.7 kDa [9].

The b subunit of Shigatoxin binds to specific glycolipid globotriaosylceramide receptors (Gb3), which are expressed at the surface of glomerular, endothelial, mesangial, and tubular cells. Specific damage of these target cells and tissue is most likely due to upregulation of Gb3 receptor expression. The subunit of Shigatoxin is modified by partial proteolysis and inhibits protein synthesis at the level of peptide translation and in addition Shigatoxin induces apoptosis of target cells. Consequently, pathological changes, like damage of the endothelium of the kidney, can result in thrombus formation [10]. In addition, novel regulatory effects of Shigatoxin as a complement modulator were recently reported. Shigatoxin binds the complement inhibitor Factor H and modulates the regulatory functions of this central human complement inhibitor [11].

Atypical hemolytic uremic syndrome

About 10 % of HUS patients develop the atypical form (aHUS), which is distinct and different from the typical HUS form and which occurs both in families and spontaneously [1, 2]. This atypical form of HUS (aHUS) is frequent in adults (>20 years), has a poor prognosis, and terminal renal insufficiency occurs in over 50% of the patients with death rates close to 25%. Recent publications also report infection to be a triggering factor for aHUS, including *Bordetella pertussis* and *Varicella* infections [12, 13]. About 20% of cases of aHUS have a familial background. This form of HUS is associated with genetic defects in particular mutations of complement genes. In 1991 two brothers were reported who both developed aHUS based on a congenital defect for complement Factor H, the major regulator of the alternative complement pathway [14]. In the meantime over 100 different aHUS-associated mutations have been reported within the Factor H gene [15]. Factor H is a human plasma protein composed of 20 homologous domains (Fig. 2a). Factor H controls complement activation on the level of the C3 convertase, by competing with complement factor B for binding to C3b, acting as a cofactor for the serine protease complement Factor I to inactivate newly formed C3b molecules or enhancing the dissociation of the C3 convertase [16]. The major complement regulatory functions of Factor H are mediated by the N-terminus of the protein by domains SCRs 1–4. The C-terminal part, i.e., SCRs 18–20 of Factor H, include the central surface attachment region and this C-terminal region also binds to C3b, to endothelial cells, to basement membranes, and to glycosaminoglycans [17]. Most of the aHUS-associated Factor H mutations that have been identified, which in most cases present in a heterozygous form, are located within the C-terminal region and result in reduced surface binding of Factor H (Fig. 2a) [15]. In particular, during conditions of local complement activation and immune stress, defective surface binding results in deregulation of complement and in enhanced complement activation. aHUS is a disease associated with inappropriate or defective complement regulation. Besides Factor H gene mutations, additional genes can be affected in aHUS that code for the complement regulators Factor I, MCP/CD46, and thrombomodulin, and for the two major components that form the C3 convertase of the alternative pathway, C3 and Factor B. This suggests that an inappropriate activity of the C3 convertase, by gain of function, mutations or by uncontrolled regulation, results in pathophysiology (Fig. 3).

Recently, a novel link between the complement and the coagulation system was identified in aHUS. Mutations in the gene coding for thrombomodulin in patients and families with aHUS were described [18]. Thrombomodulin is a central regulator of blood coagulation that controls and regulates thrombin activation and promotes inactivation of C3a and C5a, two anaphylatoxins that are generated upon complement activation, and which represent central inflammatory effector compounds. These results also define a role of thrombomodulin in the alternative pathway of complement [19]. Thrombomodulin binds to the central alternative pathway regulator Factor H and to the regulator of the classical pathway C4BP. About 5% of aHUS cases are caused by mutations in the thrombomodulin gene.

A detailed follow-up study on a large French cohort of aHUS encompassing 217 patients allows conclusions for the prognosis and progression of the disease. An early disease onset correlates with less likelihood of kidney failure and a lower mortality. Juvenile patients had more relapses and benefit more from plasma therapy. Genetic defects also correlate with disease onset and

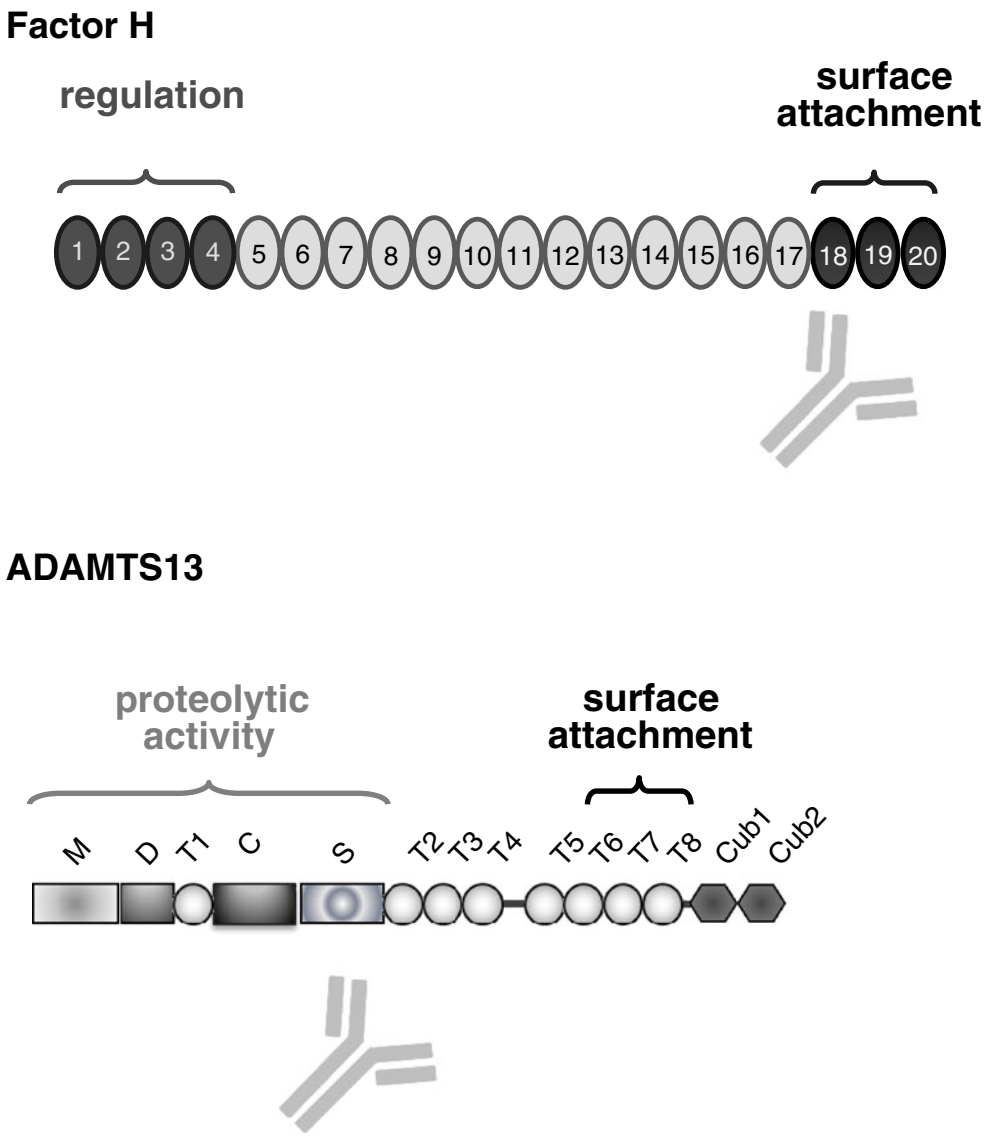

Fig. 2 Genetic and acquired factors in HUS and TTP. **a** Domain structure of Factor H and binding site of the autoantibodies in DEAP-HUS. Factor H is composed of 20 consecutive, individually folding protein domains termed short consensus repeats. The two major functional regions of Factor H are located at the opposite ends of the protein. The N-terminal SCRS1-4 represents the regulatory region, which mediates complement regulatory effects on the level of the C3 convertase, C3bBb. The C-terminal SCRs 18–20 form the surface attachment region, which makes contact with cell surfaces, in particular the surface of damaged cells, with C3b and C3d, and with glycosaminoglycans. The aHUS-associated gene mutations cluster in particular in the C-terminal surface attachment region of Factor H. Similarly, the autoantibodies associated with DEAP-HUS bind to the C-terminus of Factor H and block surface attachment. Thus, autoantibodies block and the C terminal gene mutations also affect surface recognition activity of Factor H. **b** Domain structure of *ADAMTS13* and preferred binding site of autoantibodies. *ADAMTS13* (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) has a modular composition, being formed of M: metalloprotease domain, D: disintegrin-like domain, T1: thrombospondin type 1 repeat, C: Cys-rich region, S: spacer region, and CUB-like domains 1 and 2. The position of the two major functional regions that form the proteolytic (MDT1CS) and surface attachment region (T6T7T8) are indicated. The binding sites for the autoantibody are also shown

mutations in the membrane cofactor protein (MCP), autoantibodies for Factor H, and combined defects are more often observed in patients with early disease onset. This analysis shows the relevance of screening for genetic mutations and for autoantibodies for prognosis and therapy of aHUS [20].

The autoimmune form of HUS: DEAP-HUS

A third form of HUS, termed DEAP-HUS (deficient for CFHR genes and autoantibody positive), was recently identified [4, 21]. This form is based on two conditions, the presence of an acquired factor in the form of autoantibodies

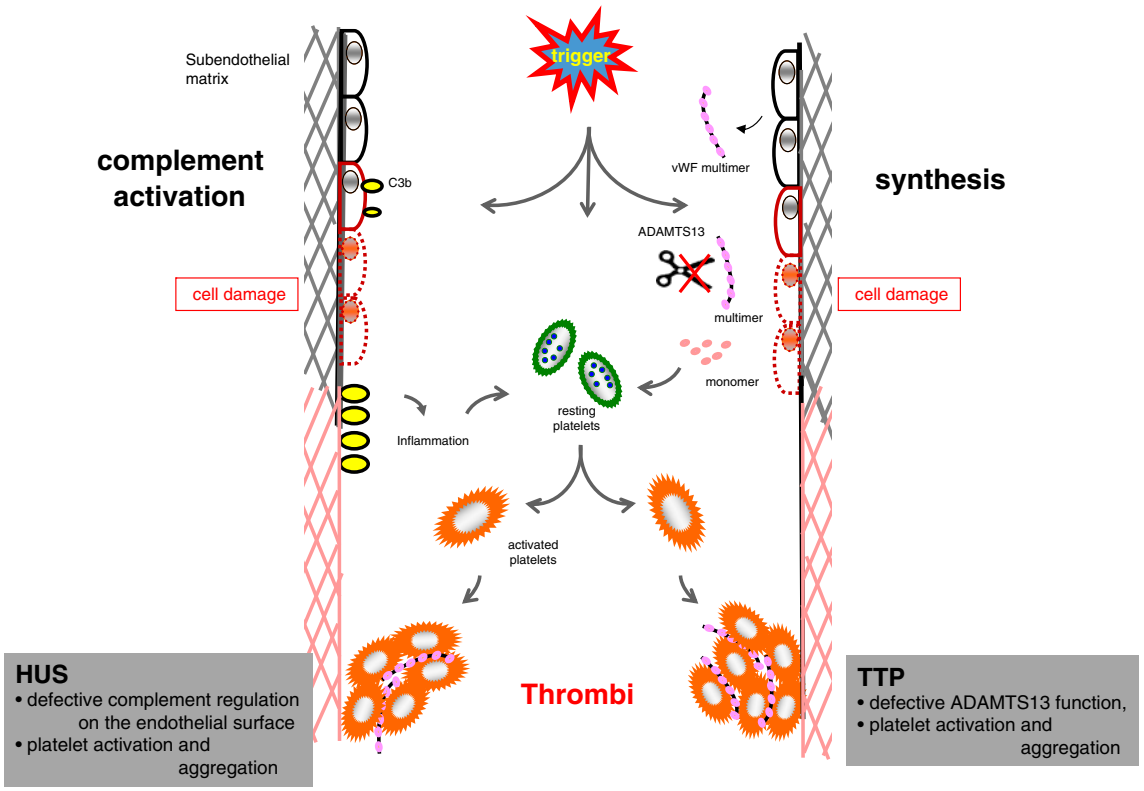

Fig. 3 A common link for the pathophysiology of HUS and TTP: defective complement regulation vs inappropriate processing of vWF multimers. An initial trigger in the micro vessels can cause local activation of the complement and of the coagulation cascade. *Left*: Complement activation and newly generated activation products may attack and damage host endothelial cells, which leads to exposure of the subendothelial matrix. Inappropriate complement control and/or enhanced action of the C3 convertase can further amplify the cascade, increasing local damage, which is further transferred to the surface of the platelets. This can activate the platelets, leading to clotting and thrombus formation. *Right*: A rather similar scenario can explain thrombus formation in TTP. Multimers of von Willebrand factor (vWF) are released, but processing is impaired by a defective *ADAMST13* or by an enzyme that is inactivated by autoantibodies. As a consequence, the large vWF multimers accumulate, bind to the surface of the platelets, activate the platelets, and induce thrombus formation

that in DEAP-HUS develop on a genetic defect in the form of a deletion of a large chromosomal fragment in the CFHR gene cluster, encompassing the CFHR1 and CFHR3 genes [22–24]. As most of the DEAP-HUS patients are aged approximately 6–16 years, this group is considered a new entity distinct from aHUS, where genetic alterations are predominantly observed in adult individuals (>20 years).

This autoimmune form is identified in about 10% of HUS patients. Most of the disease-associated autoantibodies bind to the C-terminus of Factor H and reduce Factor H binding to C3b and to cell surfaces. Thus, the autoantibodies in DEAP-HUS and the C-terminal mutations in the Factor H gene described in aHUS have very similar and related effects. In both cases the C terminal Factor H functions and recognition is impaired [22]. The consequence of the blocking autoantibodies or Factor H C-terminal mutations is uncontrolled local complement activation that leads to inflammation, cell damage, and platelet activation.

The genetic analyses of the Jena DEAP-HUS cohort showed that most patients lack a chromosomal 85-kbp fragment that includes the CFHR1 and CFHR3 genes and patients develop autoantibodies [23, 24]. This concept was confirmed in other groups like the Spanish- and the Newcastle cohorts [25]. Based on these conditions DEAP-HUS patients require a novel form of diagnosis and therapy. For most patients treatment with therapeutically B-cell targeting antibodies or plasma exchange combined with immunosuppressive treatment lowered autoantibody titers. This kind of therapy improved the conditions of DEAP-HUS patients and was also relevant for kidney transplantation. A reduction of autoantibody titers prior to transplantation lowered the risk of disease recurrence in the transplant [26, 27]. Owing to the lack of a standardized approved protocol for DEAP-HUS therapy novel approaches are being followed that are mostly based on clinical experience in the treatment of related autoimmune disorders. Several DEAP-HUS patients responded to

immunosuppressive therapy, plasma exchange, and steroids [27]. However, a second group of patients did not respond to this immunosuppressive treatment.

Genetics of HUS

Genetic analyses identified in about 50–65% of aHUS patients mutations in complement genes, such as the regulators Factor H, Factor I, MCP (CD46) as well as C3 and Factor B, which form the central complement convertase of the alternative pathway C3bBb [28]. In addition, deletion of the genes coding for CFHR1 and CFHR3, as well as hybrid Factor H proteins, have been linked to HUS.

Factor H gene mutations have also been reported in MPGN patients (membranoproliferative glomerulonephritis, also termed dense deposit diseases). Based on this similarity and despite substantial clinical differences, it was hypothesized that related genetic scenarios may underlie the two diseases [29]. Thus, mutated complement genes may affect the complement activation and regulation on different levels, which may progress either to HUS or MPGN [30]. However, the individual genetic background per se does not allow a precise prediction for one or the other of the clinical diseases. Thus, HUS and MPGN may represent different spectral outcomes of a common disease principle. Based on this hypothesis HUS, in particular during the first years of life, may have a more severe and acute progression compared with MPGN, which shows a more chronic progression. About 50–65 % of HUS patients have a known genetic defect in the form of mutations in central complement regulatory proteins or components that form the C3 convertase (C3bBb) of the alternative pathway of complement. In contrast, in MPGN, the fraction of known genetic defects is smaller and so far accounts for about 10–15% of the cases. Also, most HUS-associated mutations are heterozygous, in particular Factor H gene mutations. MPGN-associated mutations in the Factor H gene mostly affect both alleles and represent either homozygous or compound heterozygous variations [30].

The complement network provides protection of renal cells platelets and surfaces in particular of the glomerular basement membrane. As defective complement action and regulation lead to kidney diseases, detailed functional characterization of each component, and also of the interplay of these components, is of central relevance. Several examples show how genetic and functional defects of single complement components lead to defective complement action and represent a source of autoimmune diseases [31]. Examples are more frequent diseases such as systemic lupus erythematosus (SLE), nephritis or vasculitis [32]. One feature of these three disorders is reduced clearance of apoptotic cells and of apoptotic particles [33]. Such a reduced clearance results in accumulation of particles and their exposure to antigen-presenting immune cells [34]. This may lead to the generation of autoantibodies against self structures and ultimately in autoimmune diseases. These examples underline the requirement of knowledge about genetic deficiencies and functional defects in the clearance mechanisms to diagnose and follow a structured therapy for such autoimmune diseases.

Therapy for HUS

Therapy for typical HUS, atypical HUS, and DEAP-HUS should follow the guidelines described by the various European or British consortia. In addition, the different genetic and other profiles are relevant for a prognosis for the outcome of kidney transplantations and also for the risk of disease recurrence after kidney transplantation or combined liver and kidney transplantations.

In addition, novel therapeutic options are urgently needed for treatment of HUS patients. Unrestricted and uncontrolled complement activation leads to generation of the inflammatory anaphylatoxin C5a and formation of the terminal complement complex. Therefore, targeting and inhibiting complement at the level of the C5 convertase seems a very promising approach for therapy. Novel therapeutic antibodies or fusion proteins have been established over the last few years and are now used for HUS therapy. For example, the C5 targeting humanized antibody, eculizumab (Soliris®), is a promising candidate for controlling complement in clinical settings [35]. Eculizumab was initially licensed to treat patients suffering from paroxysmal nocturnal hemoglobinuria (PNH) [36]. PNH is caused by a genetic defect that causes defective membrane integration of GPI-anchored proteins. PNH patients lack the terminal complement inhibitors CD59 and CD46/MCP on the membranes of erythrocytes and consequently in PNH erythrocytes become susceptible to complement-mediated damage. Eculizumab reduced the number of thrombolytic insults, the need for transplantation, and improved the quality of life of the patients. Eculizumab was also used to treat HUS patients and the initial results are positive and rather promising [37]. However, apparently single patients did not respond to this complement inhibitor [38].

Targeting the complement cascade is a rather promising concept and additional novel therapeutic compounds are being developed. One of these new inhibitors for the alternative complement pathway, termed TT30, is a fusion protein of the C3 binding region of the complement receptor 2 linked to the regulatory domains of Factor H, to SCRs 1–4 [39, 40]. This fusion protein CR2-Factor H has a relatively long biological half-life and is a rather efficient inhibitor of the alternative complement pathway.

Patients treated with complement inhibitors are at risk of increased microbial infection, in particular meningococcal

infections, and therefore it is recommended to vaccinate patients prior to the treatment.

Thrombotic thrombocytopenic purpura

Thrombotic thrombocytopenic purpura (TTP) is a disease that occurs in young and adult patients and is more frequent in female than in male subjects. Thrombocytopenia and purpura manifest together with microangiopathic hemolytic anemia and fragmentocytes, i.e., damaged erythrocytes [41]. The disease is associated with neurological symptoms and also with defective kidney functions. TTP as a form of TMA was originally reported in 1924 by Moschcowitz [42]. About 20 years ago in 1990, Moake identified unusually large multimers of a coagulation molecule, the von Willebrand Factor (vWF), in sera of patients with idiopathic TTP [43]. Immunohistological assays with microthrombi from TTP patients showed deposits composed of platelets and vWF and a low abundance of fibrin and fibrinogen deposits [44]. vWF is normally produced and secreted as large complexes, termed ultra-large von Willebrand polymers, which are proteolytically processed in the circulation to smaller subunits [45]. Under shear-stress conditions these ultra-large von Willebrand multimers show a higher affinity to platelets and they bind with high affinity to the platelet glycoprotein 1Bα. In contrast, the smaller, processed subunits do not bind to the platelet surface. In 1998 Furlan and coworkers [45] and also Tsai and Lian [46] independently reported that patients with acute idiopathic, non-familial TTP have inhibitory antibodies against the protease that cleaves and dissociates the ultra-large von Willebrand multimers [46, 47]. In addition, another group of patients was identified that showed a genetic defect [48]. The 6 patients with familial TTP, who lacked proteolytic processing by the von Willebrand cleaving protease, had genetic defects in the gene coding for *ADAMTS13* [47]. *ADAMTS13* is a metalloprotease that cleaves these ultra-large multimers of the von Willebrand factor by cleaving the peptid bound between tyrosine 842 und methionine 843 within the vWF protein (Fig. 2b) [49, 50].

Patients with idiopathic TTP are positive for an IgG-autoantibody, which binds to the active region of *ADAMTS13*, mainly the Cys-rich region and the spacer domain (Fig. 2b). Antibody titers differ during the acute phase of the disease and during remission. Thus, the idiopathic form of TTP is an autoimmune disease where autoantibodies are formed against *ADAMTS13* that block the proteolytic action of the metalloprotease. This blockade of protein function results in the accumulation of ultra-large multimers of vWF and ultimately in the formation of microthrombi (Fig. 3). This concept is translated into therapy as patients are treated with rituximab, a therapeutic immunomodulatory and B cell-blocking antibody [49]. Treatment with rituximab was successful for several patients and resulted in a reduction of plasma cells in the circulation and a reduction of autoantibody titers [51, 52].

Novel therapeutic approaches and disease markers are being developed for TMA. From a British registry, which includes patients with acquired TTP and with typical symptoms such as neurological insults, seizures, and renal insufficiency, the effect of the B cell targeting mAB rituximab was evaluated. At the time of admission more than 50% of the patients showed elevated troponin T levels that correlated with disease severity and mortality [53]. As autoantibodies are associated with TTP the concept was to use rituximab in order to suppress autoantibody-producing B cells and to lower both frequency and severity of relapses. Patients treated with rituximab in combination with steroids required less frequent plasma exchanges. During a period of 25 months all patients receiving rituximab remained in remission, whereas 50% of the control group suffered a relapse. Rituximab administered upon the initial increase in *ADAMTS13* autoantibody titers in patients with known TTP prevented disease relapse, thus demonstrating a preemptive effect and a good approach as therapy.

In addition, novel approaches based on aptamers or nano-bodies are being evaluated for treating TTP. The aptamer ARC1779, which specifically binds to the vWF receptor on the platelet surface inhibits vWF-mediated platelet adhesion [54]. Initial pharmacological results with this aptamer sound promising and show an anti-coagulant effect upon inhibition of vWF. This aptamer also antagonizes the increased platelet adhesion mediated by ultra-large von Willebrand factor multimers that are released in TTP. In a clinical study -this aptamer- was used for the treatment of a patient with therapy refractory TTP with high titers of ADAMTS13-antibodies and decreased platelet count. Application of this antibody reversed the clinical situation. A phase II clinical study in eight TTP patients showed no severe side effects and no bleeding events. Exceptions were hypersensitivity reactions. Five patients who finished the treatment protocol are healthy. Thus, inhibition of platelet reactivity against vWF is a promising candidate for the treatment of TTP [54]. Also, at least in an animal model of TTP, new approaches like cell-mediated gene therapy have so far shown promising effects [55].

Common features and novel therapeutic approaches

Based on these novel data, new genetic causes, and knowledge, common features of the disease mechanisms, in particular, the effect on platelet function, are emerging.

Platelets play a major role in thrombotic microangiopathies and defective platelet function and deregulated clot formation is central for the two major forms of TMA. Microthrombi from D+HUS patients stain positive for fibrinogen and thrombin and have low levels of vWF. This is in contrast to thrombi of TTP patients, which exhibit predominantly vWF [56]. Shigatoxin, the virulence factor derived from enterohemorrhagic *E. coli* (EHEC) causes complex formation of platelets and leukocytes. This consequently induces the release of tissue factor-containing micro-particles from activated human blood cells. In addition, Shigatoxin induces C3 deposition on platelet-monocyte, and platelet-neutrophil complexes, which results in progression of complement activation leading further to C9 deposition and TCC formation. This type of platelet activation may contribute to the thrombotic event [56–58].

Autoantibodies are associated with TMA and with other autoimmune diseases.

Although the exact mechanism that leads to the generation of autoantibodies in TMA needs to be worked out, one open issue is whether TMA-associated autoantibody formation is based on the same/or related principles to that established for the common autoimmune disease systemic lupus erythematosus (SLE). In SLE autoantibodies are generated that react with several "self-antigens," such as DNA and immune complexes. A detailed understanding of how defective B-lymphocyte stimulation and activation result in the generation of autoantibodies is also central to therapy. Antibody-producing B cells can be targeted by the therapeutic antibody rituximab, which binds to the specific surface marker CD20 and causes depletion of the B cell pool. In the SLE patients, therapy with rituximab was effective and reduced autoantibody titers. However, cases were reported in which rituximab was not efficient.

Thrombotic microangiopathies also occur in other diseases and recent studies have also shown evidence of complement activation in the form of high plasma levels of complement activation products and increased *ADAMTS13* activity in patients with preeclampsia. A comparison of a cohort of 60 pregnant preeclampsia patients with a control group representing 57 healthy pregnant women in the third trimester of pregnancy revealed higher levels of systemic complement activation for women with preeclampsia compared with members of the control group. Future experiments are directed toward a more detailed characterization of complement activation and regulation of this form of the disease [59]

Outlook

Diseases associated with thrombus formation due to deregulation of complement and coagulation represent a challenging field for both clinicians and basic researchers. This fast development in this interesting area of translational medicine defines on the one hand novel disease-associated genes as well as new pathophysiological principles, and on the other had it also identifies new links between immunological networks, such as the crosstalk between the complement and the coagulation cascades. The characterization of these complex interactions defines new parameters for diagnosis and therapy, and therefore provides a benefit for the patients in the form of new diagnostic markers and new approaches to therapy.

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