review

memo (2018) 11:227–234
https://doi.org/10.1007/s12254-017-0380-y

memo
magazine of european medical oncology

Role of complement in the pathogenesis of thrombotic microangiopathies

Eszter Trojnár · Ágnes Szilágyi · Bálint Mikes · Dorottya Csuka · György Sinkovits · Zoltán Prohászka

Received: 3 November 2017 / Accepted: 15 December 2017 / Published online: 17 January 2018

Summary Thrombotic microangiopathies (TMAs) are rare but life-threatening disorders characterized by microvascular hemolytic anemia and acute thrombocytopenia with or without organ damage. The term TMA covers various subgroups of diseases, the pathogenesis of which is briefly summarized in this review. As highlighted here, complement activation may represent an important amalgamating process in all of these conditions, since it is able to link together activation and damage of multiple involved cell types, such as endothelial cells, platelets, and neutrophils.

Keywords Hemolytic uremic syndrome · Thrombotic thrombocytopenic purpura · ADAMTS13 · Shiga toxin · Alternative pathway

Abbreviations

ADAMTS13	A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
ADAMTS13	The gene encoding a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13
aHUS	Atypical hemolytic uremic syndrome
AP	Alternative pathway
C3	The gene encoding the complement component C3
CD46	The gene encoding the membrane cofactor protein
CFB	The gene encoding complement factor B
CFH	The gene encoding complement factor H
CFHR1	The gene encoding the complement factor H related protein-1
CFHR5	The gene encoding the complement factor H related protein-5
CFI	The gene encoding complement factor I
DAF	Decay-accelerating factor
DGKE	Diacylglycerol kinase epsilon
DGKE	The gene encoding diacylglycerol kinase epsilon
E. coli	*Escherichia coli*
ET-1	Endothelin-1
F1+2	Plasma prothrombin fragment 1+2
Gb3	Globotriaosylceramide
HSCT	Hematopoietic stem cell transplantation
HUS	Hemolytic uremic syndrome
L-FABP-1	Liver fatty acid binding protein-1
LPS	Lipopolysaccharide
MCP	Membrane cofactor protein
NET	Neutrophil extracellular trap
sC5b-9	Soluble C5b-9 complex, or membrane attack complex
STEC-HUS	Hemolytic uremic syndrome in connection to Shiga toxin-producing *Escherichia coli* infection
sTNFR1	Soluble tumor necrosis factor receptor-1
Strep.	*Streptococcus pneumoniae*
Stx	Shiga toxin
sVCAM1	Soluble vascular cell adhesion molecule-1
T-antigen	Thomsen-Friedenreich antigen
THBD	The gene encoding thrombomodulin
TIMP-1	Tissue inhibitor of metalloproteinases-1
TMA	Thrombotic microangiopathy
TTP	Thrombotic thrombocytopenic purpura
ULVWF	Ultra-large form of von Willebrand factor
WPB	Weibel-Palade body

E. Trojnár · Á. Szilágyi · B. Mikes · D. Csuka · G. Sinkovits · Prof. Z. Prohászka (✉)
George Füst Complement Diagnostic and Research Laboratory, 3rd Department of Internal Medicine, Semmelweis University, Budapest, Hungary
prohaszka.zoltan@med.semmelweis-univ.hu

The complement system

The complement system is a main branch of the humoral immune response that plays an important role in the elimination of pathogens and altered self-structures. It may be activated through three distinct pathways: the classical, lectin and the alternative pathways. The former two are triggered by pattern recognition receptors bound to antibodies or surface-carbohydrate structures, while the alternative pathway (AP) is continuously activated by a low-rate self-activation of the C3 molecule. The three activation cascades converge on the level of C3 which is cleaved into the C3a and C3b fragments by the C3-convertase complexes. The C3b fragment attaches to foreign surfaces, initiates further C3 activation and subsequent terminal pathway activation, which causes inflammation and tissue damage (Fig. 1). Since the AP is continuously activated, its inefficient regulation may lead to overactivation of the complement system with substantial endothelial injury, such as seen in thrombotic microangiopathies (TMAs).

Forms of TMA

TMAs are rare but life-threatening disorders characterized by microvascular hemolytic anemia and acute thrombocytopenia with or without organ damage (for example signs of neurological or kidney injury). While the bed-side, immediate diagnosis of TMA is based on the clinical picture and routine laboratory results, it may cover various subgroups of diseases, the pathogenesis of which is shortly summarized in Table 1. Despite similarities in the initial presentation, TMA differential diagnostics has an impact on both long-term patient follow-up, and the determination of the optimal therapeutic choice from early on. Hemolytic uremic syndrome (HUS) usually presents with the triad of hemolytic anemia, thrombocytopenia and acute renal failure (a minority of patients may suffer from neurologic symptoms as well). Typical HUS (90–95% of the cases) is preceded by a gastrointestinal infection with Shiga-toxin-producing bacteria, and it represents the most common cause of acute kidney failure in children. Atypical HUS (aHUS) may also present with gastrointestinal symptoms that could be misleading at the initial phase; however, endothelial injury in aHUS mainly results from the dysregulation of the alternative complement pathway.

Patients with thrombotic thrombocytopenic purpura (TTP) usually show characteristic clinical symptoms such as critical thrombocytopenia and microangiopathic hemolytic anemia along with neurological symptoms, mental status changes, or sometimes signs of kidney injury. All are severe conditions that require immediate attention and diagnostic workup for complement abnormalities. To underline the characteristics of these subgroups, their distinct pathogenic features are detailed in this review, with emphasis on the role of complement (Table 1) and on the potential novel biomarkers (Table 2) of these diseases.

Complement mediated atypical hemolytic uremic syndrome (aHUS)

A number of different alterations in the complement genes encoding activators and regulators of the AP are known to be directly associated with relapsing (atypical) HUS. Since the first description of a causative factor H mutation [1], numerous further disease-causing mutations have been described in the complement genes, such as *CFH*, *CFI*, *CD46*, *C3*, *CFB*, *THBD* and *CFHR5* [2–4]. All these pathogenic variants account for around 50–60% of aHUS cases [4] but in the remainder of patients no disease-causing variations can be identified. This observation is consistent with the fact that the combined presence of a genetic predisposition and environmental trigger factors is needed to provoke an aHUS episode [3]. Pathogenic aHUS-associated mutations in the complement regulator genes lead to overactivation of the AP. *CFH* mutations mostly affect the C-terminus of the protein, which is responsible for binding to surface-associated C3b and self-specific glycosaminoglycans. Hence, the altered regulator is unable to recognize its targets and to act as a cofactor of factor I mediated C3b degradation (Fig. 1). This results in impaired complement regulation on host cell surfaces and, upon a triggering event, may lead to extensive C3b deposition on the vascular endothelium, thus, inducing cellular injury and microvascular thrombosis. Disease-causing mutations in *CD46* mainly account for reduced expression or decreased binding to C3b, whereas mutations in *CFI* lead to impaired C3b inactivation. On the other hand, gain-of-function mutations in *C3* or *CFB* result in a hyperactive, regulation-resistant C3-convertase.

It is noteworthy that the mutation penetrance in pedigrees is incomplete, which supports the hypothesis that the additive effect of multiple mutations and risk variations is often necessary to cause a clinically manifest disease. This together with the high number of patients with unidentified disease-associated genetic alterations highlights the fact that additional, yet unexplored genetic abnormalities may also contribute to the development of aHUS.

Genetic predisposing factors are also present in the autoimmune form of aHUS, where autoantibodies directed against factor H are the key pathogenic factors of disease manifestation. This TMA subgroup is strongly associated with the polymorphic homozygous deletion of the complement factor H-related gene *CFHR1* [5].

STEC-HUS

Diarrhea-associated or typical HUS is the most common form of HUS. The condition is usually evoked by a Shiga toxin (Stx)-producing *Escherichia coli* (STEC)

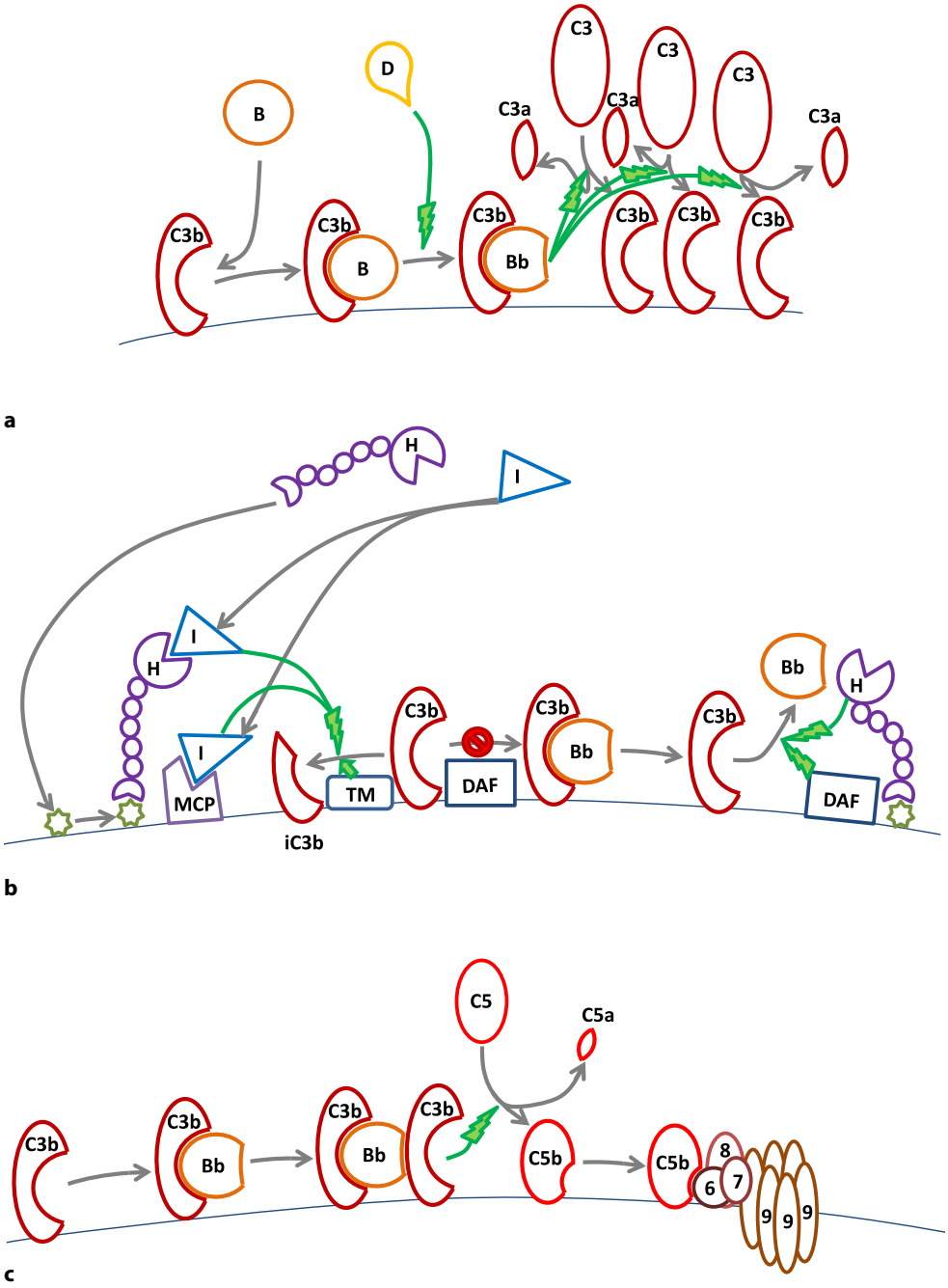

Fig. 1 Basic mechanisms of alternative pathway activation and complement regulation. **a** Complement fragment C3b binds to surfaces and factor B, which is then cleaved by factor D. The newly formed C3bBb complex (alternative pathway C3-convertase) is able to cleave multiple further C3 molecules. Thus, the alternative pathway is not only capable of spontaneous activation, but can enhance complement activation triggered through any pathway, functioning as a positive feedback loop. **b** Human surfaces are protected from the detrimental effects of complement activation by numerous surface-bound (MCP, DAF, thrombomodulin) and soluble (factor H and I) regulators. Factor I cleaves and inactivates C3b in the presence of its cofactors, factor H and MCP; thrombomodulin potentiates C3b cleavage. Factor H binds to sialic acid present on human cell membranes, but not on most pathogens, and serves as a cofactor for factor I. In addition, factor H and DAF facilitate the decay of the C3-convertases. **c** If the above regulation is defective, excess C3b binds to C3-convertases, turning them into C5-convertases, which cleave C5 to C5a and C5b, initiating the common terminal pathway. C5a is a potent anaphylatoxin (similar to C3a), while surface-bound C5b assembles the C6-9 molecules, forming a pore in the membrane, called the membrane attack complex. *MCP* membrane cofactor protein, *DAF* decay accelerating factor, *THBD* thrombomodulin. Complement factors are marked by letters. Complement components C6–C9 are marked by numbers only

Table 1 Key pathogenetic factors and the potential role of complement in various TMA forms

TMA subgroup	Key pathogenetic factor	Role of complement in the pathogenesis
Complement mediated atypical HUS	Endothelial damage due to severe dysregulation of the complement AP	Genetic alteration in certain complement genes and subsequently altered expression/function of their encoded proteins and/or loss of complement regulatory function of factor H due to autoantibodies directed against the protein
DGKE-aHUS	Loss of DGKE function results in a prothrombotic state on endothelium	sC5b-9 may alter podocyte metabolic pathways, the structure and function of the extracellular matrix, membrane lipids and key proteins of the cytoskeleton and slit-diaphragm contributing to podocyte damage in this form of TMA
Cobalamin C deficiency associated HUS	Impaired metabolism of the dietary vitamin B12 and subsequent accumulation of metabolic intermediates	Not known
STEC-HUS	Endothelial damage caused by the binding and internalization of Stx, which inhibits intracellular protein synthesis	Complement activation through Stx and LPS Increased C3b binding to the endothelium due to high P-selectin expression induced by Stx
Strep. pneumoniae/Influenza induced HUS	Neuraminidase production and cleavage of N-acetylneuraminic acid from glycoproteins on the cell membrane of erythrocytes, platelets, glomeruli and hepatocytes, exposure of the Thomsen-Friedenreich (T) antigen	Interaction of the T-antigen with preformed IgM initiates excessive complement activation of both the classical and alternative pathways and results in direct endothelial injury
Acquired TTP	Inhibitory antibodies that block the ADATMS13 metalloproteinase	C3b binding and complement activation through activated endothelial cells and platelets Direct activation of the complement AP by the ULVWF
Inherited TTP (Upshaw Schulman Syndrome)	Congenital deficiency of the ADATMS13 metalloproteinase due to mutations in *ADATMS13* that alter protein expression and/or function	
Secondary TMA	Worsening of a known preexisting condition (sepsis, solid organ or HSC transplantation, tumor progression, systemic autoimmune disease, etc.) and subsequent coagulopathy, and tissue or organ damage including the endothelium	Dysregulation of both the classical and alternative pathways with severe consumption of the individual complement factors

TMA thrombotic microangiopathy, *HSC* hematopoietic stem cell, *TTP* thrombotic thrombocytopenic purpura, *ULVWF* ultra-large form of von Willebrand factor, *HUS* hemolytic uremic syndrome, *aHUS* atypical hemolytic uremic syndrome, *STEC-HUS* hemolytic uremic syndrome in connection to Shiga toxin-producing Escherichia coli infection, *LPS* lipopolysaccharide, *DGKE* diacylglycerol kinase epsilon, *AP* alternative pathway, *Stx* Shiga toxin, *IgM* immunoglobulin M

infection. Its initial symptoms are related to the bacterial colonization of the gastrointestinal tract causing intestinal inflammation and—often bloody—diarrhea. Stx1 and Stx2 released by the adhered bacteria are the primary cause of microangiopathy through their globotriaosylceramide (Gb3) receptor mediated internalization and blockade of protein synthesis within the endothelial cells [6].

Accordingly, STEC-HUS is primarily not a complement-mediated disorder; however, increased levels of the complement-degradation products C3a(desArg), C3d, Bb, C3bBbP and sC5b-9, detected in the circulation during the acute phase of the disease provide clear evidence for an increased complement activity in this form of HUS [7–9]. This can most probably be attributed to the direct or indirect effects of Stx and bacterial lipopolysaccharide (LPS) on complement activation and coagulation. In vitro studies demonstrated that Stx can activate the alternative pathway in the fluid phase, while upon binding to the surface recognition sites of factor H, Stx may delay its inhibitory effect and promote complement activation on the cellular surface [10]. Besides, Stx—particularly in the presence of LPS—was shown to induce the formation of platelet–leukocyte aggregates and the release of blood cell-derived microparticles coated with C3 and C9 [8, 11]. Furthermore, Stx was shown to promote the upregulation of the membrane adhesion molecule P-selectin on microvascular endothelium, which—by acting as a C3b-binding protein—increases C3 deposits and favors platelet thrombus formation, thus increasing the circulatory C3a level [12]. Involvement of the alternative pathway in the microvascular processes was also supported by *in vivo* experiments: Thrombotic effects of Stx/LPS treatment could be diminished in factor B-deficient mice or could be inhibited by the admission of a C3a receptor antagonist [12] in animal models of STEC-HUS.

Thrombotic thrombocytopenic purpura (TTP)

TTP is caused by the deficiency of the ADAMTS13. The role of ADAMTS13 is to cleave the ultra-large form of von Willebrand factor (ULVWF), which is secreted by activated endothelial cells, and can spontaneously bind and activate platelets.

ADAMTS13 deficiency is necessary, but not enough, to provoke TTP, since ADAMTS13 deficiency may also be present in its convalescence. Similarly to aHUS, the onset of TTP is often associated with a triggering event. Endothelial activating conditions like pregnancy or infections may cause expression of ULVWF from endothelial cells, which, in combination with ADAMTS13 deficiency, may result in the increased presence of ULVWF molecules and consequent initiation of platelet thrombus formation. Activated platelets and endothelial cells express P-selectin, which is able to bind C3b and activate the complement system [13]. ULVWF is also able to directly bind complement factors and trigger complement al-

Table 2 Characteristic laboratory findings and potential biomarkers of various TMA forms

TMA subgroup	Characteristic laboratory markers or disease-causing factors	Potential biomarkers and novel prognostic markers of disease severity
Complement mediated atypical HUS	Mutation(s) and/or risk variation(s) in genes encoding complement components (C3, factor B) or regulators (factor H, I, MCP, *THBD*, CFHR5) in most of the patients	Decreased alternative pathway activity, C3 and factor B level Elevated plasma levels of Ba, sTNFR1, sVCAM1, thrombomodulin, F1+2, D-dimer [23] Elevated urine concentrations of C5a, sC5b-9, clusterin, TIMP-1, cystatin-C, L-FABP-1 [23]
Complement mediated atypical HUS, autoimmune	Presence of anti-factor H autoantibodies Homozygous deletion of *CFHR1* (in ~90% of the cases)	Decreased alternative pathway activity, C3 and factor B level Decreased factor H level
DGKE-aHUS	Mutations present in the *DGKE* gene	Presence of proteinuria [24]
Cobalamin C deficiency associated HUS	Normal plasma vitamin B12 and folate level Increased total homocysteine but normal or decreased methionine level in plasma Increased plasma or urinary methylmalonate level	Not known
STEC-HUS	*E. coli* producing Stx Positive *E. coli* serology	Blood urea nitrogen to serum creatinine ratio [25] Decreased neutrophil extracellular trap degradation [26] Neutrophil gelatinase-associated lipocalin [27]
Strep. pneumoniae/ Influenza induced HUS	Presence of invasive S. pneumoniae infection Proof of neuraminidase activity (T-antigen conversion)	Decreased classical and alternative pathway activities, C3 and C4 level Systemic neuraminidase activity [28, 29]
Acquired TTP	ADAMTS13 activity <10% Inhibitory autoantibodies against ADATMS13	Circulating endothelial cells, soluble P-selectin, ULVWF Carboxiterminal-pro-endothelin-1[30] Elevated plasma neutrophil elastase concentration [17] Higher IgA, IgG1 and IgG3 anti-ADAMTS13 antibodies [31]
Inherited TTP (USS)	ADAMTS13 activity <10%	Not known
Secondary TMA	Combined consumption of complement factors and decreased activity of both the classical and alternative pathways Level of activation markers may be increased (sC5b-9; C3a)	Various prognostic markers depending on the type of background disease (e.g. double-stranded DNA in transplantation associated TMA) [20]

TMA thrombotic microangiopathy, *TTP* thrombotic thrombocytopenic purpura, *ULVWF* ultra-large form of von Willebrand factor, *HUS* hemolytic uremic syndrome, *LPS* lipopolysaccharide, *DGKE* diacylglycerol kinase epsilon, *AP* alternative pathway, *MCP* membrane cofactor protein, *Stx* Shiga toxin, *USS* Upshaw–Schulman syndrome, *Stec* Shiga toxin-producing Escherichia coli, *IgM* immunoglobulin M
MCP membrane cofactor protein, *THBD* thrombomodulin, *IgA* immunoglobulin A, *IgG1* immunoglobulin G1 subclass, *IgG3* immunoglobulin G3 subclass

ternative and terminal pathway activation [14], thus, leading to increased levels of complement activation products C3a, C5a, and sC5b9 in acute phase TTP patients [15, 16]. These complement activation products further activate endothelial cells, initiating the vicious circle of increased ULVWF and P-selectin expression, and decreased thrombomodulin expression [14]. Furthermore, complement activation leads to granulocyte activation [17] and subsequent cellular adherence to the endothelium facilitated by the increased P-selectin expression. Production of reactive oxygen species and proteases by the attached granulocytes further enhances endothelial dysfunction [18]. In summary, complement activation is part of an amplification loop: it augments the prothrombotic changes in the microvasculature that leads to a full-blown thrombotic microangiopathy (Fig. 2).

Secondary TMA

Secondary forms of TMA represent a heterogeneous group of disorders that all emerge on the basis of a preexisting condition. Secondary TMA may be associated with infections and septic conditions, allogenic hematopoietic stem cell (HSC) or solid organ transplantation, systemic autoimmune diseases, pregnancy, tumor progression or malignant hypertension. Even though their etiology may vary, it is common in secondary TMA that overactivation and subsequent consumption of both classical and alternative pathway complement components, and decreased ADAMTS13 activity are present in these conditions.

The involvement of the complement alternative pathway dysregulation has been recently suggested in the pathogenesis of post-HSCT-TMA. In this condition, an elevated systemic sC5b-9 level was associated with worse long-term outcome [19]. *Gloude et al.* also suggested that chemotherapy, radiation and infections leading to endothelial injury during HSCT provoke complement activation through neutrophil activation and neutrophil extracellular trap (NET) release [20, 21]. In line with these findings, increased activation of all three complement pathways was observed in our series of secondary TMA patients with various etiologies and elevated sC5b-9 and C3a concentrations were associated with a poor patient outcome [22]. Although the pathophysiology of these TMA forms has not entirely been explored, the clear involvement of complement may support future plans to study complement inhibitors such as eculizumab in these conditions.

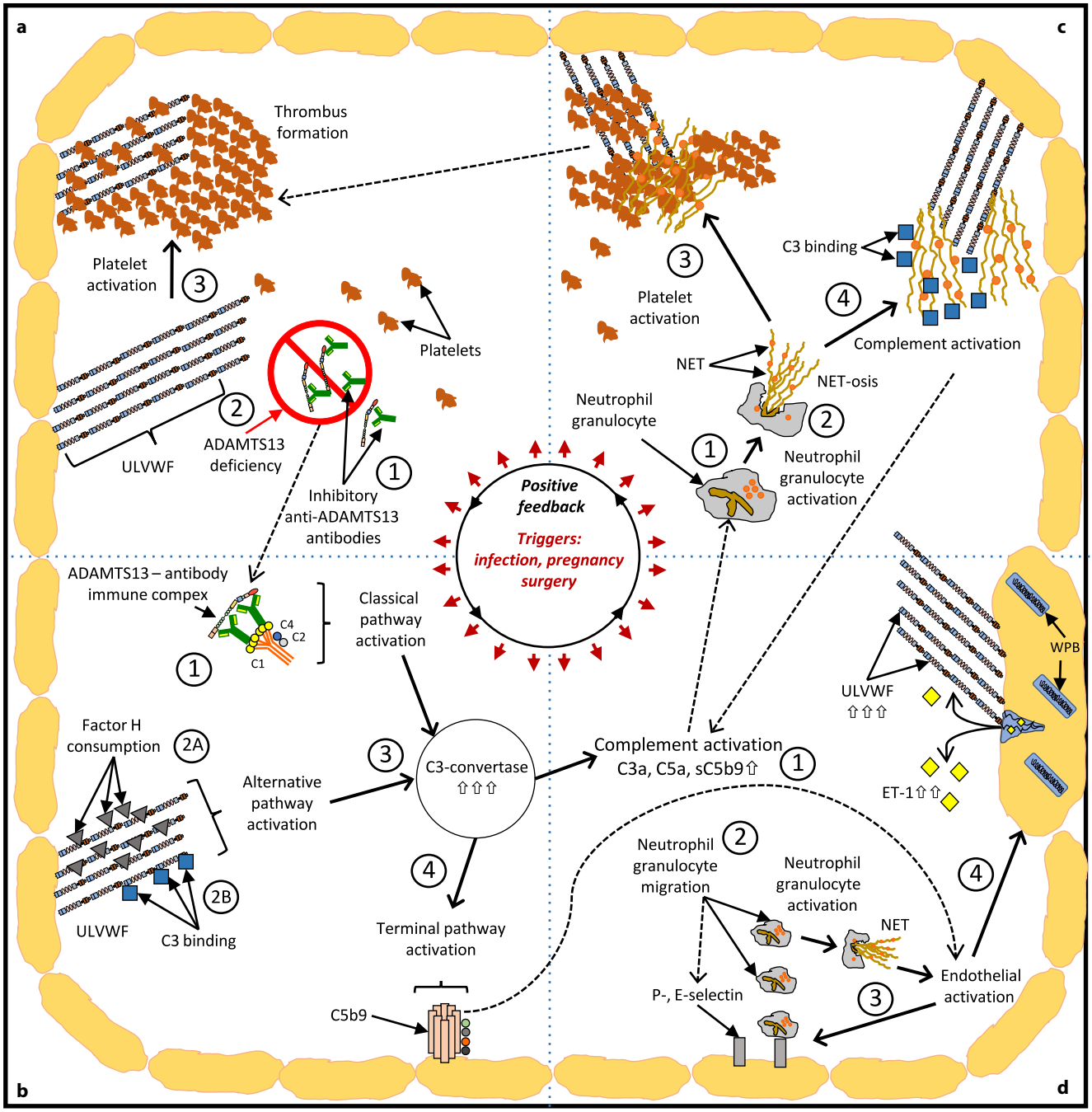

Fig. 2 Summary of thrombotic microangiopathy (TMA) pathogenesis. **a** In case of ADAMTS13 deficiency, uncleaved ultra-large form of von Willebrand factor (*ULVWF*) provides a surface for platelet aggregation and thrombus formation. **b** Antibody–antigen complexes, ULVWF, and other molecules on activated endothelial cells and platelets trigger complement activation. **c**, **d** The activated terminal pathway can in turn activate neutrophil granulocytes and facilitate their binding to endothelial cells, leading to endothelial injury and prothrombotic changes in the endothelium. The activated neutrophil cells can release neutrophil extracellular traps (*NET*), which provides a surface for thrombus formation and complement activation. The above events are present to a different extent in distinct forms of TMA, with the complement system connecting them to form a vicious circle. *Numbers in circles* indicate the order of events in each section. *C5b-9* C5b-9 complex, or membrane attack complex, *ET-1* endothelin-1, *WPB* Weibel-Palade body

Discussion

Our understanding of the pathophysiology and characteristic course of various TMA forms has improved in recent years with novel genes, pathways and mechanisms described as a result of intensive research of this field. As highlighted in this review, complement activation may represent an important amalgamating process in all of these conditions, since it is able to link activation and damage of multiple involved cell types, such as endothelial cells, platelets, and neutrophils (Fig. 2). The recent knowledge on the pathophysiology of TMAs was translated into clinical use and has reached the clinical care of patients, too, since more and more laboratories provide appropriate tests for clinical diagnostics (Table 1). In addition, future research will help to clarify if biomarkers of the above described pathways (Table 2) are appropriate tools for prediction of disease exacerbation or severity.

The current management of various TMAs largely relies on supportive care, infection control, immunosuppression, cytostatics and therapeutic plasma exchange. There are only a few targeted therapies available for TMA patients that include B-cell depletion by the anti-CD20 monoclonal antibody rituximab, inhibition of platelet adhesion by caplacizumab, a nanobody targeting von Willebrand factor administered in TTP or complement inhibition with anti-C5 monoclonal antibody eculizumab for patients with aHUS. The accumulated knowledge on the role of neutrophils, endothelial cells, platelets, and the complement system in the pathophysiology of TMAs may open new avenues for research on additional targeted therapies, including the blockage of neutrophil activation and degranulation with colchicine, inhibition of complement activation (for example with drugs limiting C3 activation and alternative pathway amplification) or its action (such as C5a receptor blockade), or preparations that restore endothelial function.

Conflict of interest E. Trojnár, Á. Szilágyi, B. Mikes, D. Csuka, G. Sinkovits, and Z. Prohászka declare that they have no competing interests.

References

1. Warwicker P, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998;53(4):836–44.
2. Szarvas N, et al. Genetic analysis and functional characterization of novel mutations in a series of patients with atypical hemolytic uremic syndrome. Mol Immunol. 2016;71:10–22.
3. Caprioli J, et al. Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. Blood. 2006;108(4):1267–79.
4. Westra D, et al. Atypical hemolytic uremic syndrome and genetic aberrations in the complement factor H-related 5 gene. J Hum Genet. 2012;57(7):459–64.
5. Jozsi M, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood. 2008;111(3):1512–4.
6. Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. Microbiol Spectr. 2014; https://doi.org/10.1128/microbiolspec.EHEC-0024-2013.
7. Thurman JM, et al. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2009;4(12):1920–4.
8. Stahl AL, Sartz L, Karpman D. Complement activation on platelet-leukocyte complexes and microparticles in enterohemorrhagic Escherichia coli-induced hemolytic uremic syndrome. Blood. 2011;117(20):5503–13.
9. Westra D, et al. Serological and genetic complement alterations in infection-induced and complement-mediated hemolytic uremic syndrome. Pediatr Nephrol. 2017;32(2):297–309.
10. Orth D, et al. Shiga toxin activates complement and binds factor H: evidence for an active role of complement in hemolytic uremic syndrome. J Immunol. 2009;182(10):6394–400.
11. Arvidsson I, et al. Shiga toxin-induced complement-mediated hemolysis and release of complement-coated red blood cell-derived microvesicles in hemolytic uremic syndrome. J Immunol. 2015;194(5):2309–18.
12. Morigi M, et al. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. J Immunol. 2011;187(1):172–80.
13. Del Conde I, et al. Platelet activation leads to activation and propagation of the complement system. J Exp Med. 2005;201(6):871–9.
14. Bettoni S, et al. Interaction between multimeric von Willebrand factor and complement: a fresh look to the pathophysiology of microvascular thrombosis. J Immunol. 2017;199(3):1021–40.
15. Reti M, et al. Complement activation in thrombotic thrombocytopenic purpura. J Thromb Haemost. 2012;10(5):791–8.
16. Westwood JP, et al. Complement and cytokine response in acute Thrombotic Thrombocytopenic Purpura. Br J Haematol. 2014;164(6):858–66.
17. Mikes B, et al. Elevated plasma neutrophil elastase concentration is associated with disease activity in patients with thrombotic thrombocytopenic purpura. Thromb Res. 2014;133(4):616–21.
18. Ruiz-Torres MP, et al. Complement activation: the missing link between ADAMTS-13 deficiency and microvascular thrombosis of thrombotic microangiopathies. Thromb Haemost. 2005;93(3):443–52.
19. Jodele S, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood. 2013;122(12):2003–7.
20. Gloude NJ, et al. Circulating dsDNA, endothelial injury, and complement activation in thrombotic microangiopathy and GVHD. Blood. 2017;130(10):1259–66.
21. Yuen J, et al. NETosing neutrophils activate complement both on their own NETs and bacteria via alternative and non-alternative pathways. Front Immunol. 2016;7:137.
22. Farkas P, et al. Complement activation, inflammation and relative ADAMTS13 deficiency in secondary thrombotic microangiopathies. Immunobiology. 2017;222(2):119–27.
23. Cofiell R, et al. Eculizumab reduces complement activation, inflammation, endothelial damage, thrombosis, and renal injury markers in aHUS. Blood. 2015;125(21):3253–62.

24. Azukaitis K, et al. The phenotypic spectrum of nephropathies associated with mutations in diacylglycerol kinase epsilon. J Am Soc Nephrol. 2017;28(10):3066–75.
25. Balestracci A, et al. Blood urea nitrogen to serum creatinine ratio as a prognostic factor in diarrhea-associated hemolytic uremic syndrome: a validation study. Eur J Pediatr. 2017; https://doi.org/10.1007/s00431-017-2999-4.
26. Leffler J, et al. Decreased neutrophil extracellular trap degradation in Shiga toxin-associated haemolytic uraemic syndrome. J Innate Immun. 2017;9(1):12–21.
27. Lukasz A, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) in patients with Shiga toxin mediated haemolytic uraemic syndrome (STEC-HUS). Thromb Haemost. 2014;111(2):365–72.
28. Huang DT, et al. T-antigen activation for prediction of pneumococcus-induced hemolytic uremic syndrome and hemolytic anemia. Pediatr Infect Dis J. 2006;25(7):608–10.
29. Szilagyi A, et al. The use of a rapid fluorogenic neuraminidase assay to differentiate acute streptococcus pneumoniae-associated hemolytic uremic syndrome (HUS) from other forms of HUS. Clin Chem Lab Med. 2015;53(4):e117–e9.
30. Mikes B, et al. Carboxiterminal pro-endothelin-1 as an endothelial cell biomarker in thrombotic thrombocytopenic purpura. Thromb Haemost. 2016;115(5):1034–43.
31. Bettoni G, et al. ADAMTS-13 activity and autoantibodies classes and subclasses as prognostic predictors in acquired thrombotic thrombocytopenic purpura. J Thromb Haemost. 2012;10(8):1556–65.