

Chapter 2

Complement Interactions with Blood Cells, Endothelial Cells and Microvesicles in Thrombotic and Inflammatory Conditions

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Abstract The complement system is activated in the vasculature during thrombotic and inflammatory conditions. Activation may be associated with chronic inflammation on the endothelial surface leading to complement deposition. Complement mutations allow uninhibited complement activation to occur on platelets, neutrophils, monocytes, and aggregates thereof, as well as on red blood cells and endothelial cells. Furthermore, complement activation on the cells leads to the shedding of cell derived-microvesicles that may express complement and tissue factor thus promoting inflammation and thrombosis. Complement deposition on red blood cells triggers hemolysis and the release of red blood cell-derived microvesicles that are prothrombotic. Microvesicles are small membrane vesicles ranging from 0.1 to 1 μm , shed by cells during activation, injury and/or apoptosis that express components of the parent cell. Microvesicles are released during inflammatory and vascular conditions. The repertoire of inflammatory markers on endothelial cell-derived microvesicles shed during inflammation is large and includes complement. These circulating microvesicles may reflect the ongoing inflammatory process but may also contribute to its propagation. This overview will describe complement activation on blood and endothelial cells and the release of microvesicles from these cells during hemolytic uremic syndrome, thrombotic thrombocytopenic purpura and vasculitis, clinical conditions associated with enhanced thrombosis and inflammation.

Keywords Complement • Microvesicles • Platelets • Neutrophils • Monocytes • Red blood cells • Endothelial cells • Hemolytic uremic syndrome • Thrombotic thrombocytopenic purpura • Vasculitis

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2.1 Complement Activation in Thrombotic and Inflammatory Disease

The complement system is an assembly of proteins activated via three pathways, the classical, lectin and alternative pathways [1]. All ultimately result in activation of the common terminal complement pathway. Complement activation results in disposal of foreign cells, such as bacterial pathogens, unwanted host cells, such as apoptotic cells, or immune complexes, by opsonization or cytolysis. Certain complement components have anaphylatoxic, anti-microbial and chemotactic properties [1]. Due to its potent effects the complement system needs to be strictly regulated to prevent uncontrolled activation on host cells. Even so, complement may become activated, with secondary harmful effects, during infectious, hereditary and autoimmune diseases.

Complement activation may be so extensive that regulators are overwhelmed thus allowing continued activity. Alternatively, if the regulators are dysfunctional or the complement components are hyperfunctional, complement activation may proceed in an uninhibited manner. Regardless of the cause, inappropriate activation will be deleterious to host cells in the vasculature at the interface of blood cells and the endothelium. Activation on blood cells and endothelial cells will predispose the host to thrombosis, inflammation and hemolysis by rendering the endothelium pro-thrombotic while activating platelets, leukocytes and red blood cells. Activated blood and endothelial cells may shed microvesicles [2].

2.2 Complement Interaction with the Endothelium

Complement activation occurs on endothelial cells during inflammatory and thrombotic processes and enhances cell injury as well as vascular permeability and leukocyte recruitment to the vascular wall and underlying tissues. In infectious and inflammatory conditions the endothelium may be markedly affected, by bacterial virulence factors, platelet microthrombi, as well by neutrophil proteases and migration. Complement activation is either a primary or secondary phenomenon. Endothelial cells express complement factors, regulators and their receptors [3, 4]. Endothelial complement deposition will lead to cell activation, expression of adhesion molecules [5, 6], release of cytokines and chemokines [7–10], membrane attack complex (MAC) formation [11] and ultimately cytolysis. C5b-9 formation on the endothelial cell induces von Willebrand factor (VWF) secretion and expression of the catalytic surface for the prothrombinase enzyme complex as well as shedding of microvesicles with assembled C5b-9 on their surfaces [12]. C3a and C5a are released activation products that induce cytokine release [13, 14]. C5a and IL-8 (also secreted by the endothelium) are chemotactic for polymorphonuclear leukocytes. Vascular permeability is increased by complement activation [15] in part due to C3a and C5a [16] and the terminal complement complex, C5b-9, which also induces increased

endothelial permeability [17] followed by leukocyte migration into the extravascular space [18]. Tissue factor release by endothelial cells is mediated by IL-1 α and regulated by complement [19] promoting a procoagulant phenotype.

2.3 Microvesicles

Microvesicles are extracellular organelles that have an important role in inflammatory and thrombotic processes. These plasma membrane vesicles are shed from cells during activation or apoptosis [20]. Complement deposition on cells may trigger microvesicle shedding [21–23]. Microvesicles are larger (100–1000 nm) than exosomes and are not preformed within the cell's multivesicular bodies but rather shed directly from the cell membrane by the process of ectocytosis [21]. The phospholipid bilayer plasma membrane of these vesicles exposes receptors from the parent cell, and the cytosolic content is similarly derived from the cell of origin. Microvesicles can communicate with cells in their immediate vicinity or at remote sites from the parent cell by receptor binding of the entire microvesicle, by detachment of a protein from the microvesicle that serves as a ligand binding to the target cell, by fusion of the microvesicle to the cell or by endocytosis [24]. This interaction may lead to a signal cascade affecting essential cell functions such as proliferation, differentiation and apoptosis [25]. Increased numbers of circulating microvesicles occur in thrombotic and inflammatory diseases [20].

Most microvesicles in the systemic circulation originate from platelets [26] but they may originate from a variety of cells, including leukocytes [27], red blood cells [28] and endothelial cells [29]. As they transport substances derived from the cell of origin they may contain a broad spectrum of receptors, DNA, mRNA, micro RNA, histones and other proteins, as well as lipids capable of interacting with cells.

Of specific importance are the proinflammatory and prothrombotic properties of circulating microvesicles. Leukocyte-derived microvesicles harbor complement, chemokines, cytokines, proteases, HLA antigens, and adhesion molecules [27]. Upon contact these microvesicles can activate resting platelets and endothelial cells. Endothelial cells respond by expression of adhesion molecules [30]. Platelet-derived microvesicles bind to endothelial cells and induce the synthesis of pro-inflammatory cytokines and arachidonic acid pathway products [31]. In the context of inflammation, they also promote binding of monocytes to endothelial cells [32]. Furthermore, platelet microvesicles modulate adaptive immunity by affecting immunoglobulin production by B cells [33]. Endothelial-derived microvesicles have been shown to express markers involved in cellular adhesion and inflammation such as CD62E (E-selectin), CD62P (P-selectin), ICAM-1 (intercellular adhesion molecule-1), integrin $\alpha_v\beta_3$, CD31 (PECAM-1: platelet endothelial cell adhesion molecule-1), CD105 (endoglin), CD144 (VE-cadherin), VWF and CD146 (S-endo-1) [34]. Microvesicles released from cytokine-stimulated endothelial cells induced secretion of soluble ICAM-1 from targeted endothelial cells [35]. Endothelial microvesicles may be considered biomarkers of sustained endothelial activation [23].

The prothrombotic potential of microvesicles is mostly related to their exposure of phosphatidylserine and tissue factor. The release of microvesicles by cell activation is a calcium-dependent enzymatic process, involving scramblase, floppase and translocase/flippase activities [36, 37], leading to disruption of phospholipid membrane asymmetry during which phosphatidylserine is translocated to the outer membrane of the released microvesicles [20, 37, 38]. Not all microvesicles expose phosphatidylserine on the outer leaflet [38]. Phosphatidylserine can bind coagulation factors VII, IX, X, and prothrombin thus initiating clotting. Microvesicles from platelets were shown to have a higher procoagulant potential than the parent cell [39]. The prothrombotic effect was also demonstrated in red blood cell-derived microvesicles [40].

Tissue factor is the receptor for factor VIIa and initiates the extrinsic coagulation cascade and thrombin generation. Tissue factor has been demonstrated on microvesicles derived from monocytes and endothelial cells [38] and also on platelet-derived microvesicles released from platelet-leukocyte aggregates [41]. Furthermore, tissue factor may be transferred to activated platelets on the surface of endothelial and monocytic microvesicles [34].

2.3.1 Detection of Microvesicles

Microvesicle detection requires specific techniques. The vesicles are isolated from biological fluids by a series of centrifugations and detected using flow cytometry and cell-specific markers. The use of multiple markers enables higher specificity and exclusion of contaminating subpopulations. Most microvesicles expose phosphatidylserine and will therefore bind annexin V. Their size is determined by comparison with fluorescently-labeled particles/beads of a defined size and the quantity/mL is calculated using a specified quantity of blank calibration particles [42].

In addition to flow cytometry-based methodology microvesicles can be studied using proteomics, electron microscopy, ELISA (using immobilized annexin V) as well as by nanoparticle tracking.

2.3.2 Complement Activation and Microvesicle Release in Clinical Conditions

Elevated levels of blood cell- and endothelial cell-derived microvesicles have been demonstrated in prothrombotic states such as acute coronary syndrome [43], atherosclerotic disease [44], end stage renal disease [23], hemolytic uremic syndrome (HUS) [41, 42, 45, 46], thrombotic thrombocytopenic purpura (TTP) [47, 48], autoimmune thrombocytopenia [49] and vasculitis [50]. Our current understanding of the contribution of complement activation and microvesicles to the pathogenesis of the thrombotic and inflammatory entities comprised in thrombotic microangiopathies and vasculitides will be summarized below.

2.4 Hemolytic Uremic Syndrome

HUS is defined by the simultaneous occurrence of non-immune hemolytic anemia, thrombocytopenia and acute renal failure [51]. The pathological kidney lesion, known as thrombotic microangiopathy (TMA), is characterized by vessel wall thickening, detachment of the endothelium from the basement membrane with accumulation of amorphous material in the subendothelium and formation of microthrombi in glomerular capillaries leading to vessel occlusion. This lesion is typical of both HUS and TTP although the underlying pathogenetic mechanisms differ.

HUS is subdivided based on etiology into cases associated with specific infections, or not, the latter including atypical HUS and secondary causes (Table 2.1) [52, 53]. The most common subtype of HUS is associated with enterohemorrhagic *Escherichia coli* (EHEC) infection. The second most common cause is atypical HUS, aHUS, associated with mutations in complement regulators or factors, as well as auto-antibodies to the complement regulator factor H.

Table 2.1 Classification and subtypes of hemolytic uremic syndrome (HUS)

	Cause
<i>Infection-associated</i>	
EHEC-HUS ^a	Shiga toxin-producing non-invasive strains that can cause hemorrhagic colitis: enterohemorrhagic <i>E. coli</i> (EHEC) or <i>Shigella dysenteriae</i>
<i>Streptococcus pneumoniae</i>	Neuraminidase-producing invasive strains
HIV ^b	
<i>Not associated with specific infection</i>	
aHUS ^c	Mutations in complement factors and regulators and auto-antibodies to factor H
DGKE ^d deficiency	Recessive DGKE mutations
Cobalamin metabolism defect	Cobalamin type C MMACHC mutations, methylmalonic aciduria and homocystinuria
Drugs	Quinine, mitomycin, anti-VEGF
Malignancies	Tumors and anti-cancer treatment, drugs and irradiation
Solid organ transplantation	Including use of calcineurin inhibitors
SLE ^e and antiphospholipid syndrome	
Pregnancy HELLP ^f syndrome	

^aHUS caused by bacterial strains that produce Shiga toxin and induce hemorrhagic colitis, also known as D+ HUS, diarrhea-associated
^bHIV: human immunodeficiency virus
^caHUS: atypical HUS
^dDGKE: diacylglycerol kinase ϵ
^eSLE: systemic lupus erythematosus
^fHELLP: HEmolytic anemia, elevated Liver enzymes, and Low Platelets

2.4.1 EHEC-Associated HUS: Toxin Interaction with Blood Cells, the Endothelium and Complement Activation

EHEC bacteria produce a unique virulence factor known as Shiga toxin (Stx). The strains themselves are non-invasive, thus remaining in the intestinal tract after colonization [54]. EHEC infection is associated with enteritis followed, in approximately 15 % of cases by the development of HUS [55]. The remote target organ damage occurring in the kidneys and other organs, including the central nervous system, during HUS, is secondary to systemic spread of the toxin and other bacterial virulence factors together with a potent intestinal and systemic host response [56–58]. Stx has been demonstrated in the circulation of HUS patients bound to platelets, neutrophils and monocytes [41, 59] but not in free form [60]. It has also been demonstrated in the kidneys of affected HUS patients [61, 62].

Stx binds to the glycosphingolipid receptor globotriaosylceramide (Gb3/CD77), also known as the P^k antigen on red blood cells. The Gb3 receptor is present on a variety of cells including platelets, monocytes and endothelial cells [63–65]. The toxin receptor on neutrophils has been proposed to be toll-like receptor 4 (TLR4) [66]. After binding, the holotoxin undergoes endocytosis. Intracellular toxin is transported via a retrograde route from early endosomes to the endoplasmic reticulum. From there the A subunit is translocated to rRNA where it inhibits protein synthesis leading to cell death. The cytotoxic effect has been demonstrated in renal glomerular endothelial cells [67] and tubular cells [68, 69]. However, not all cells are affected in this way. Certain cells, primarily blood cells, are activated by the toxin to secrete inflammatory mediators [58, 64, 70–72]. Platelets, in particular, when partially activated, can be further activated by Stx [41, 73, 74]. The combination of platelet activation and endothelial cell injury will promote a prothrombotic interaction and enhance inflammation.

Thrombocytopenia occurring during HUS is secondary to platelet consumption on the endothelium in the microangiopathic lesion. Both Stx and lipopolysaccharide circulate bound to platelets as well as to other blood cells [73, 75] and both virulence factors activate platelets, either directly and/or in the presence of inflammatory mediators, particularly under conditions of high shear stress [41]. Our group has shown that co-stimulation with both factors induced formation of platelet-leukocyte complexes that expressed tissue factor [41].

During HUS, platelets are activated [76] and degranulated [77, 78], as indicated by elevated platelet-derived factors such as β -thromboglobulin, platelet factor-4 and VWF [79]. The latter is also secreted from endothelial cells [80, 81] and mediates Stx-induced platelet adhesion to activated endothelial cells involving the platelet receptor GPIIb/IIIa and P-selectin [82]. Platelets may also be activated by chemokines and cytokines released by Stx-stimulated monocytes [71] or endothelial cells [72].

Platelet activation and thrombus formation during HUS are thus caused by a direct interaction of bacterial virulence factors, especially Stx, with platelets and endothelial cells, as well as an indirect inflammatory effect, reflected by cytokine release,

which further enhances the platelet-endothelial interaction [74]. Our group showed that Stx induced the formation of platelet-leukocyte aggregates coated with C3 and C9, an effect enhanced by co-stimulation with *E. coli* O157 lipopolysaccharide [83]. Platelets are capable of binding C3, C3b, C3d and C4, as well as expressing P selectin and CR2 that bind C3b and C3d, respectively [74, 84–87]. Complement activation progresses to the terminal pathway and the cytolytic membrane attack complex may be assembled on the platelet surface [22, 83]. Complement deposition on platelets has an activating prothrombotic effect on the cells [88–91]. The reverse was also shown, that the complement system can become sequentially activated on activated platelets [84]. Thus, in order to prevent excessive complement activation platelets express and bind complement regulators, including factor H [92–98]. In vitro studies have, however, shown that Stx2, at rather high concentrations, could bind to factor H at its host surface recognition domain, thus inhibiting its regulatory effect and allowing activation of the alternative pathway of complement in serum [99].

Components of the coagulation cascade are not consumed as a consequence of the thrombotic process occurring during HUS, but prothrombin fragment 1+2 [100–102], tissue plasminogen activator, tissue plasminogen activator inhibitor-1 [103], and D dimers are elevated suggesting prothrombotic activity and impaired fibrinolysis.

In addition to platelets, neutrophils and monocytes, Stx can bind to red blood cells via the P^k antigen (an antigen within the PIPK blood group system) present on all red blood cells except for the rare p phenotype [42, 104]. Binding was shown, by our group, to induce hemolysis as indicated by release of lactic dehydrogenase and hemoglobin [42]. The hemolytic effect of Stx was complement-mediated, via the alternative pathway, as it was reduced in the absence of plasma and when plasma was heat-inactivated as well as in the presence of EDTA or eculizumab, a humanized anti-C5 antibody.

Stx has been shown to have a direct cytotoxic effect on endothelial cells [105] including glomerular endothelial cells. This effect may be enhanced in the presence of proinflammatory cytokines such as interleukin 1 and tumor necrosis factor [65, 105]. The toxin induces the expression of P selectin on microvascular endothelial cells. P selectin binds C3, thus activating the alternative pathway and reducing thrombomodulin. This process was found to promote thrombus formation under perfusion in vitro, and in a mouse model in vivo [106].

The induction of endothelial cell injury and microthrombi formation in glomeruli alongside severe tubular damage [68] would be expected to induce secondary complement activation during EHEC-mediated HUS. This is indeed the case as patients have been shown to have low levels of serum C3 [107, 108] and elevated levels of complement products C3a, factor Bb and soluble C5b-9 [83, 109], as well as complement C3 and C9 on platelet-leukocyte aggregates suggesting that bacterial virulence factors and the host response induce complement activation on these blood cells. C3 has also been demonstrated on patient red blood cells [42] implicating a role in the induction of hemolysis.

Taken together, the findings in patients, animal models and in vitro studies indicate that complement is activated on blood cells and the endothelium during

EHEC-associated HUS and that Stx plays a major role in this process. Complement activation could aggravate the procoagulant interaction in the vasculature and thus contribute to formation of the pathological lesion manifest in thrombotic microangiopathy.

2.4.2 Microvesicles in EHEC-HUS

Patients with EHEC-associated HUS have elevated levels of circulating microvesicles derived from platelets, monocytes, neutrophils [41, 83, 110] and red blood cells [42]. Plasma microvesicle concentrations were higher during the acute phase of disease, decreasing to normal levels after recovery. The microvesicles exposed phosphatidylserine and a large proportion bore tissue factor [41], which could trigger thrombosis. In addition, blood cell-derived microvesicles had deposits of C3 and C9 [42, 83], suggesting that complement was either activated on the parent cells before the vesicle bud off, or occurred directly on the microvesicle. Complement deposition contributed to opsonization of labeled microvesicles [83]. A recent finding was that microvesicles circulating in EHEC-HUS patients bore Stx which could thus be transferred to kidney cells and evade immune detection [46].

In vitro studies showed that Stx and lipopolysaccharide from *E. coli* O157:H7 induced the release of blood cell-derived microvesicles, mostly from platelets but also from monocytes. These microvesicles exhibit deposits of tissue factor [41], C3 and C9 [83] as well as Stx [46]. Stx also triggered the release of C5b-9 coated red blood cell-derived microvesicles, an effect mediated by complement activation via the alternative pathway and inhibited by purinergic receptor antagonists [42]. Thus the findings in patients could be reproduced in vitro by stimulation with Stx.

2.4.3 Atypical HUS: Complement Interactions with Blood Cells and the Endothelium

aHUS is in many cases familial and recurrent leading to renal failure and vascular complications [111, 112]. It has been associated with mutations in complement regulators factor H, factor I, membrane cofactor protein (MCP/CD46), thrombomodulin, clusterin, or complement factors C3 or factor B [111, 113, 114]. Mutations are mostly heterozygous. Their impact on protein function is described in Table 2.2. Less than 10 % of patients have mutations in more than one complement factor. A subset of patients have auto-antibodies to factor H [115], which are, in some cases, associated with deletions or rearrangements in factor H and factor H-related proteins (CFHRs) resulting in hybrid genes but usually not associated with other complement mutations.

Table 2.2 The effect of complement mutations in aHUS on protein function

Complement factor	Pathway	Soluble or membrane-bound	Complement factor or regulator	Function	Consequence of mutation in aHUS
Factor H	Alternative	Soluble	Regulator	<ul style="list-style-type: none">• Cofactor for factor I in C3b cleavage• Accelerates decay of the C3 convertase• Host cell recognition• Inhibits the C5 convertase	Enhanced complement activation on host cells
Factor H related protein-I	Terminal	Soluble	Regulator		Increased complement activation via the terminal pathway
Factor I	Alternative and classical	Soluble	Regulator	<ul style="list-style-type: none">• Cleaves C3b to iC3b (inactive form) in the presence of cofactors: factor H, C4 binding protein, MCP or complement receptor 1	Enhanced complement activation
MCP (CD46)	Alternative, classical or terminal	Membrane-bound	Regulator	<ul style="list-style-type: none">• Cofactor for factor I-mediated C3b cleavage	Enhanced complement activation
Thrombomodulin	Alternative, classical or terminal	Membrane-bound	Regulator	<ul style="list-style-type: none">• Enhanced factor I C3b cleavage with cofactor factor H• Generates TAFI that inactivates C3a and C5a• Inhibits MAC formation	Enhanced complement activation and more circulating C3a and C5a
Clusterin	Terminal	Soluble	Regulator	<ul style="list-style-type: none">• Inhibits MAC formation	Enhanced MAC formation
C3	All	Soluble	Factor	<ul style="list-style-type: none">• C3 cleavage to C3a and C3b having anaphylactic, chemotactic and anti-microbial properties• C3b forms the C3 convertase with factor B and further binding forms the C5 convertase• C3b and its inactive form iC3b are opsonins	Hyperfunctional C3 forms a stable convertase which may be resistant to degradation
Factor B	Alternative	Soluble	Factor	<ul style="list-style-type: none">• Binds to C3 and is cleaved by factor D to form the C3 convertase C3bBb	Hyperfunctional C3 convertase

MCP: membrane co-factor protein. TAFI: thrombin-activatable fibrinolysis inhibitor. MAC: membrane attack complex

Mutations in complement regulators lead to loss-of-function of the regulators thus allowing uninhibited complement activation to occur on endothelial cells [116] and platelets [45] *in vitro*. Mutations in, and antibodies to, factor H mainly affect the C terminus [117], which is involved in host recognition, differentiating host cells from foreign surfaces. Neutralization of host recognition results in undesirable complement activation on host cells. Mutations in complement factors C3 and factor B may lead to gain-of-function resulting in a hyperfunctional C3 convertase [118–120]. Thus mutations trigger complement activation, which, in patients, results in complement deposition on renal endothelial cells [121] and platelets [45, 114, 120]. Complement activation occurs via the alternative pathway followed by activation of the terminal complement cascade with deposition of C3 and C9 [45]. Complement activation occurs both systemically and locally in the renal vasculature.

The effect of aHUS mutations on interactions with blood cells and the endothelium has been studied *in vitro* and in animal models. Factor H was shown to bind to platelets mostly via its C terminal domain [96]. An aHUS mutant form of factor H, FH-E1198Stop, mutated in the C terminus, exhibited reduced binding [45] and promoted C3 deposition on normal washed platelets. Likewise, patient sera, containing mutated forms of factor H or C3, allowed deposition of both C3 and C9 on normal washed platelets leading to their activation [45, 120].

In addition to direct complement activation on platelet surfaces, platelet activation during aHUS may occur due to complement-mediated endothelial damage [116]. Uninhibited complement activation on endothelial cells has been shown to occur in the presence of mutated factor H [122], C3 and factor B mutations [118, 119] due to decreased regulatory capacity of factor H or hyperfunctional C3 or factor B leading to excess formation of the C3 convertase which may also be resistant to decay [123]. Glomerular endothelial cells stimulated with pro-inflammatory mediators (TNF and IFN γ) and exposed to aHUS serum with hyperfunctional C3 expressed tissue factor and a prothrombotic phenotype [118]. Similarly, cytokine-stimulated human umbilical vein endothelial cells exposed to certain factor B mutations bound more C3 [112, 123]. These *in vitro* studies exemplify how mutated over-functional complement mediated excessive complement deposition on the endothelium. An *in vivo* model of aHUS was developed in mice transgenically expressing a factor H protein lacking the C terminal region. These mice exhibited C3 and C9 staining in glomeruli. Thrombotic microangiopathy did not develop in mice that, in addition to mutant factor H, lacked C5, indicating that the terminal complement pathway was involved in formation of the specific glomerular lesion [124].

It would seem likely that complement should deposit on red blood cells during aHUS, as complement deposition has been demonstrated on platelets and endothelial cells. This aspect, and if complement mediates hemolysis during aHUS, has, however, not been studied. Nonetheless, once hemolysis has occurred and hemoglobin is released, heme is liberated and induces complement activation [125]. Heme was shown to activate complement via the alternative pathway in serum as well as on endothelial cells *in vitro*. The interaction between heme and C3 resulted in an overactive C3 convertase [125].

2.4.4 Microvesicles in aHUS

Sera from aHUS patients with defined factor H mutations located at the C terminal domain induced the release of tissue factor-bearing and phosphatidylserine exposing microvesicles from normal washed platelets [45]. This effect was markedly decreased when the normal platelets were preincubated with normal factor H before addition of the patient sera indicating that the mutated complement regulator contributed to shedding of prothrombotic microvesicles. Although complement deposition, secondary to excessive complement activation, has been demonstrated on blood and endothelial cells in aHUS, this has yet to be demonstrated on patient microvesicles.

2.5 Thrombotic Thrombocytopenic Purpura

TTP is defined as the simultaneous occurrence of hemolytic anemia, thrombocytopenia, renal and neurological manifestations and fever. It may be recurrent. TTP is associated with dysfunctional or deficient ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) known as the VWF cleaving protease. Deficiency of the protease occurs in congenital TTP due to compound heterozygous or homozygous mutations in the protein whereas acquired dysfunction of the protease occurs due to the presence of circulating auto-antibodies to the protease [126]. Deficiency or dysfunction of ADAMTS13 results in uncleaved ultra-large VWF multimers with high biological potency to bind platelets and form thrombi, thus predisposing patients to a prothrombotic condition. Lack of ADAMTS13 promotes the formation of platelet-VWF strings on endothelial cells in vitro [127].

The primary pathogenetic event during TTP is the formation of microthrombi and the typical microangiopathic lesion. However, even during TTP secondary complement activation occurs, which may enhance the detrimental interaction between endothelial cells and platelets. Patients with TTP, both congenital and acquired, exhibit complement activation as shown by low C3 [128], elevated C3a, C5a and C5b-9 in the circulation during acute episodes [129, 130]. Renal, skin and cardiac tissue from TTP patients exhibited complement deposition [48, 131–133]. Sera from TTP patients induced C3 deposition and MAC formation on microvascular endothelial cells and promoted neutrophil-induced endothelial cytotoxicity, effects abrogated by complement inhibition [134].

The ultra-large VWF strings formed on the endothelial surface in vitro were found to activate complement via the alternative pathway. Complement activation occurred on platelets, endothelial cells and on VWF itself under perfusion [48, 135] and the presence of ADAMTS13 abrogated this effect. Our group has shown that exposure of histamine-stimulated glomerular endothelial cells to patient plasma, but not normal plasma, induced C3 deposition on platelet-VWF strings and the endothelium [48].

Interestingly, factor H was found to bind to VWF via its C terminal region [136] and thereby affect ADAMTS13-mediated cleavage, although conflicting data have been reported suggesting it may either enhance [136], or inhibit [137], this interaction. Factor H was shown to reduce the size of VWF multimers [138] which would affect their capability to bind platelets. Moreover, VWF binding to factor H enhanced cofactor activity in conjunction with factor I [137] thus inactivating complement progression. Both factor H and VWF are secreted by endothelial cells and may thus cooperate in reducing the inflammatory and thrombotic reaction on the endothelial cell surface.

2.5.1 Microvesicles in TTP

In TTP platelet and endothelial microvesicles are released into the circulation [47, 139]. Calcium-dependent proteolytic activity (Calpain) was associated with platelet-derived microvesicles [47]. Patient endothelial microvesicles co-expressed CD62E (E-selectin) and VWF [140]. Expression of VWF may enhance the platelet aggregative potential of the microvesicles [141]. Our group has shown that endothelial microvesicles in TTP patient plasma bore deposits of C3 and C9 [48] reflecting complement activation on the vascular lining.

TTP plasma induced the release of procoagulant endothelial cell-derived microvesicles from brain and renal microvascular endothelial cells [139]. The endothelial microvesicles expressed CD62E, ICAM-1, platelet endothelial cell adhesion molecule (PECAM-1), CD105 (endoglin) and VWF [140]. In addition, TTP plasma combined with normal platelets and perfused over histamine-treated glomerular endothelial cells induced significant C3 and C9 deposition on microvesicles released from the endothelial cells, in comparison to control plasma [48].

2.6 Vasculitis

Vasculitides are a group of autoimmune diseases characterized by inflammation in and around vessel walls. They are predominantly classified according to the size of the affected vessels [142]. A subset of patients with vasculitides have anti-neutrophil-cytoplasmic-antibodies (ANCA) commonly directed to proteinase 3 and/or myeloperoxidase. Multiple organs may be affected during vasculitis, such as the kidneys, respiratory tract, gastrointestinal (GI) tract, joints and skin and thus patients may exhibit a variety of symptoms such as renal dysfunction, respiratory symptoms, sinusitis, GI-bleeding, arthritis and purpura. The clinical severity of vasculitides varies from mild and transient to life-threatening conditions, which may recur. Although the pathogenesis is not fully elucidated ANCA, complement, neutrophil- and T cell-mediated inflammation are believed to be essential in the development of disease [143–147].

The innate immune system, and especially the complement system, have been shown to play a part in the inflammatory process in vasculitis [3]. In both immune-complex

mediated vasculitides, such as in Systemic Lupus Erythematosus (SLE), and in pauci-immune vasculitides, such as ANCA-associated vasculitides, the complement system is activated [147, 148]. In SLE both immunoglobulins and complement components including C1q, C3 and C4 are deposited in the renal glomerulus, thus indicating activation of the classical pathway of complement. In addition, the importance of the classical pathway in SLE is illustrated by the association with C1q and C2 deficiency [149, 150]. On the other hand, animal models of pauci-immune vasculitides have demonstrated activation of the alternative pathway of complement [151, 152]. In these animal models C4 deficiency (classical pathway) did not affect the course of disease whereas C5 and factor B deficiency (alternative pathway) were protective suggesting that the alternative pathway mediated inflammatory injury during ANCA-related disease [153].

Neutrophil migration appears to be an essential component of tissue injury and particularly endothelial damage during ANCA-associated vasculitis. The importance of C5a, acting as a chemotactic agent and an anaphylatoxin, was demonstrated as patient neutrophils activated normal serum to produce C5a. The pivotal role of C5a was demonstrated by attenuation of the disease in a C5a receptor-deficient mouse model of necrotizing crescentic glomerulonephritis [151].

2.6.1 The Role of Microvesicles in Vasculitis

Both adults and children with vasculitis have elevated levels of microvesicles in the circulation that have been shown to correlate to disease activity [154, 155]. Endothelial-, neutrophil- and platelet-derived microvesicles have been demonstrated in vasculitis patients [50, 156]. As identification of circulating microvesicles, especially endothelial cell-derived, may reflect endothelial damage seen in vasculitis, it has been suggested that circulating microvesicles could be used as a biomarker for vasculitis activity [154, 155, 157].

Neutrophil-derived microvesicles have been shown to induce endothelial cell activation and subsequent release of cytokines [30, 158] as well as endothelial cell damage [159]. ANCA, which are present in a subset of vasculitides, have been shown to induce release of microvesicles from neutrophils in vitro, followed by microvesicle-dependent activation of endothelial cells, suggesting a pathogenic role of microvesicles in vasculitis [157].

2.7 Clinical and Therapeutic Implications of Complement Activation and Microvesicle Release

In the conditions described, HUS (both EHEC-associated and aHUS), TTP and vasculitis, complement activation on blood- and endothelial cells activates platelets, monocytes, neutrophils, red blood cells as well as endothelial cells promoting thrombotic and inflammatory damage. Activated or apoptotic cells will release

microvesicles, and the presence of complement on these microvesicles reflects the detrimental in vivo process. Once complement activation and microvesicle release manifest the vascular damage may already be, to a certain degree, irreversible. However, an ongoing continuous process may be amenable to treatment.

During EHEC infection and prior to the development of HUS antibiotic treatment may expose the patient to an increased risk of developing HUS [160]. Nonetheless, antibiotic therapy during the course of HUS may be beneficial [161]. The infection, and the renal and neurological complications that may develop, are best managed by supportive care [161, 162] as no specific treatment has, to-date, been shown to be effective. Patients exhibit excessive complement activation [42, 83, 109] and a few patients were suggested to respond to eculizumab, humanized anti-C5 antibody (Alexion, Cheshire, CT) [163], but, by the time patients present with HUS, treatment aimed at blocking complement activation may be ineffective, as reported during the large *E. coli* O104:H4 outbreak in 2011 [161, 164, 165].

In contrast, the disease process occurring during aHUS leads to continuous complement activation due to the presence of complement mutations, or antibodies to factor H. In these patients treatment with eculizumab is extremely effective, preventing disease recurrence and renal failure [166] and allowing patients who previously developed kidney failure to undergo renal transplantation [167].

Most patients with TTP are successfully treated with plasma infusions or exchange, and patients with acquired TTP respond to rituximab (anti-CD20) [126]. Complement blockade was described in one TTP patient whose disease activity was refractory to other treatments (plasma exchange, glucocorticoids, rituximab, and vincristine) [133]. Although this patient had antibodies to ADAMTS13 he was later shown to also have antibodies to factor H [168], which may explain the beneficial response to eculizumab.

Patients with vasculitis are treated with non-specific immune suppression, usually achieving remission. Eculizumab was shown to be effective in one severe pediatric case of SLE with vasculitis [169] and in an adult case of SLE complicated by thrombotic microangiopathy [170]. As animal models of necrotizing glomerulonephritis have shown that C5 is involved in the renal damage, using C5a receptor deficient mice [151], further study of complement blockade as a treatment modality in vasculitis and SLE is warranted.

In addition to the commercially available eculizumab, multiple therapeutic agents are being developed in order to block complement activation at different levels and in the different pathways [171]. These agents may prove to be promising therapeutic options for certain thrombotic microangiopathies and vasculitides in the future.

Elevated circulating microvesicles may be biomarkers of an ongoing inflammatory condition. Still, cells may attempt to eliminate unwanted substances by shedding these within microvesicles. Thus future investigations will need to address the contribution of microvesicles to the inflammatory process and if therapeutic interventions directed at blocking microvesicle release are beneficial.

2.8 Conclusions

Whether as a primary event or as a secondary phenomenon, complement activation during thrombotic and inflammatory conditions affecting the vasculature will further perturb injury to the endothelial lining. Microvesicles released from blood and endothelial cells are prothrombotic and via secretion of their contents predispose to inflammation. Conditions such as HUS, TTP and vasculitis differ in their etiologies and pathogenesis but in these conditions complement activation and microvesicle release may exacerbate the course of disease. For this reason therapeutic interventions directed at these processes are worthy of future study.

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References

1. Walport MJ. Complement. First of two parts. *N Engl J Med*. 2001;344(14):1058–66.
2. Lopez JA, del Conde I, Shrimpton CN. Receptors, rafts, and microvesicles in thrombosis and inflammation. *J Thromb Haemost*. 2005;3(8):1737–44.
3. Acosta J, Qin X, Halperin J. Complement and complement regulatory proteins as potential molecular targets for vascular diseases. *Curr Pharm Des*. 2004;10(2):203–11.
4. Langegegen H, Pausa M, Johnson E, Casarsa C, Tedesco F. The endothelium is an extrahepatic site of synthesis of the seventh component of the complement system. *Clin Exp Immunol*. 2000;121(1):69–76.
5. Lozada C, Levin RI, Huie M, Hirschhorn R, Naime D, Whitlow M, et al. Identification of C1q as the heat-labile serum cofactor required for immune complexes to stimulate endothelial expression of the adhesion molecules E-selectin and intercellular and vascular cell adhesion molecules 1. *Proc Natl Acad Sci U S A*. 1995;92(18):8378–82.
6. Kilgore KS, Shen JP, Miller BF, Ward PA, Warren JS. Enhancement by the complement membrane attack complex of tumor necrosis factor- α -induced endothelial cell expression of E-selectin and ICAM-1. *J Immunol*. 1995;155(3):1434–41.
7. van den Berg RH, Faber-Krol MC, Sim RB, Daha MR. The first subcomponent of complement, C1q, triggers the production of IL-8, IL-6, and monocyte chemoattractant peptide-1 by human umbilical vein endothelial cells. *J Immunol*. 1998;161(12):6924–30.
8. Kilgore KS, Flory CM, Miller BF, Evans VM, Warren JS. The membrane attack complex of complement induces interleukin-8 and monocyte chemoattractant protein-1 secretion from human umbilical vein endothelial cells. *Am J Pathol*. 1996;149(3):953–61.
9. Benzaquen LR, Nicholson-Weller A, Halperin JA. Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *J Exp Med*. 1994;179(3):985–92.
10. Fischetti F, Tedesco F. Cross-talk between the complement system and endothelial cells in physiologic conditions and in vascular diseases. *Autoimmunity*. 2006;39(5):417–28.

11. Hamilton KK, Ji Z, Rollins S, Stewart BH, Sims PJ. Regulatory control of the terminal complement proteins at the surface of human endothelial cells: neutralization of a C5b-9 inhibitor by antibody to CD59. *Blood*. 1990;76(12):2572–7.
12. Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. *J Biol Chem*. 1990;265(7):3809–14.
13. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *FASEB J*. 2003;17(9):1003–14.
14. Kerr H, Richards A. Complement-mediated injury and protection of endothelium: lessons from atypical haemolytic uraemic syndrome. *Immunobiology*. 2012;217(2):195–203.
15. Karpman D, Kahn R. The contact/kinin and complement systems in vasculitis. *APMIS Suppl*. 2009;127:48–54.
16. Bossi F, Peerschke EI, Ghebrehwet B, Tedesco F. Cross-talk between the complement and the kinin system in vascular permeability. *Immunol Lett*. 2011;140(1-2):7–13.
17. Bossi F, Fischetti F, Pellis V, Bulla R, Ferrero E, Mollnes TE, et al. Platelet-activating factor and kinin-dependent vascular leakage as a novel functional activity of the soluble terminal complement complex. *J Immunol*. 2004;173(11):6921–7.
18. Dobrina A, Pausa M, Fischetti F, Bulla R, Vecile E, Ferrero E, et al. Cytolytically inactive terminal complement complex causes transendothelial migration of polymorphonuclear leukocytes in vitro and in vivo. *Blood*. 2002;99(1):185–92.
19. Saadi S, Holzknacht RA, Patte CP, Stern DM, Platt JL. Complement-mediated regulation of tissue factor activity in endothelium. *J Exp Med*. 1995;182(6):1807–14.
20. Morel O, Jesel L, Freyssinet JM, Toti F. Cellular mechanisms underlying the formation of circulating microparticles. *Arterioscler Thromb Vasc Biol*. 2011;31(1):15–26.
21. Stein JM, Luzio JP. Ectocytosis caused by sublytic autologous complement attack on human neutrophils. The sorting of endogenous plasma-membrane proteins and lipids into shed vesicles. *Biochem J*. 1991;274(Pt 2):381–6.
22. Sims PJ, Faioni EM, Wiedmer T, Shattil SJ. Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J Biol Chem*. 1988;263(34):18205–12.
23. Rabelink TJ, de Boer HC, van Zonneveld AJ. Endothelial activation and circulating markers of endothelial activation in kidney disease. *Nat Rev Nephrol*. 2010;6(7):404–14.
24. Meckes Jr DG, Raab-Traub N. Microvesicles and viral infection. *J Virol*. 2011;85(24):12844–54.
25. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res*. 2010;107(9):1047–57.
26. Flaumenhaft R, Dilks JR, Richardson J, Alden E, Patel-Hett SR, Battinelli E, et al. Megakaryocyte-derived microparticles: direct visualization and distinction from platelet-derived microparticles. *Blood*. 2009;113(5):1112–21.
27. Angelillo-Scherrer A. Leukocyte-derived microparticles in vascular homeostasis. *Circ Res*. 2012;110(2):356–69.
28. Rubin O, Canellini G, Delobel J, Lion N, Tissot JD. Red blood cell microparticles: clinical relevance. *Transfus Med Hemother*. 2012;39(5):342–7.
29. Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. *Cell Tissue Res*. 2009;335(1):143–51.
30. Mesri M, Altieri DC. Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a JNK1 signaling pathway. *J Biol Chem*. 1999;274(33):23111–8.
31. Pap E, Pallinger E, Pasztoi M, Falus A. Highlights of a new type of intercellular communication: microvesicle-based information transfer. *Inflamm Res*. 2009;58(1):1–8.

32. Barry OP, Pratico D, Savani RC, FitzGerald GA. Modulation of monocyte-endothelial cell interactions by platelet microparticles. *J Clin Invest.* 1998;102(1):136–44.
33. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood.* 2008;111(10):5028–36.
34. Dignat-George F, Boulanger CM. The many faces of endothelial microparticles. *Arterioscler Thromb Vasc Biol.* 2011;31(1):27–33.
35. Curtis AM, Wilkinson PF, Gui M, Gales TL, Hu E, Edelberg JM. p38 mitogen-activated protein kinase targets the production of proinflammatory endothelial microparticles. *J Thromb Haemost.* 2009;7(4):701–9.
36. Zwaal RF, Schroit AJ. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood.* 1997;89(4):1121–32.
37. Daleke DL. Regulation of transbilayer plasma membrane phospholipid asymmetry. *J Lipid Res.* 2003;44(2):233–42.
38. Owens 3rd AP, Mackman N. Microparticles in hemostasis and thrombosis. *Circ Res.* 2011;108(10):1284–97.
39. Sinauridze EI, Kireev DA, Popenko NY, Pichugin AV, Panteleev MA, Krymskaya OV, et al. Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thromb Haemost.* 2007;97(3):425–34.
40. Rubin O, Delobel J, Prudent M, Lion N, Kohl K, Tucker EI, et al. Red blood cell-derived microparticles isolated from blood units initiate and propagate thrombin generation. *Transfusion.* 2013;53(8):1744–54.
41. Ståhl AL, Sartz L, Nelsson A, Békássy ZD, Karpman D. Shiga toxin and lipopolysaccharide induce platelet-leukocyte aggregates and tissue factor release, a thrombotic mechanism in hemolytic uremic syndrome. *PLoS One.* 2009;4(9), e6990.
42. Arvidsson I, Ståhl AL, Manea Hedström M, Kristoffersson AC, Rylander C, Westman JS, Storry JR, Olsson ML, Karpman D. Shiga toxin-induced complement-mediated hemolysis and release of complement-coated red blood cell-derived microvesicles in hemolytic uremic syndrome. *J Immunol.* 2015;194:2309–18.
43. Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation.* 2000;101(8):841–3.
44. Leroyer AS, Tedgui A, Boulanger CM. Role of microparticles in atherothrombosis. *J Intern Med.* 2008;263(5):528–37.
45. Ståhl AL, Vaziri-Sani F, Heinen S, Kristoffersson AC, Gydell KH, Raafat R, et al. Factor H dysfunction in patients with atypical hemolytic uremic syndrome contributes to complement deposition on platelets and their activation. *Blood.* 2008;111(11):5307–15.
46. Ståhl AL, Arvidsson I, Johansson K, Chromek M, Rebetz J, Loos S, Kristoffersson AC, Békássy ZD, Mörgelin M, Karpman D. A novel mechanism of bacterial toxin transfer within host blood cell-derived microvesicles. *PLoS Pathog.* 2015;11, e1004619.
47. Kelton JG, Warkentin TE, Hayward CP, Murphy WG, Moore JC. Calpain activity in patients with thrombotic thrombocytopenic purpura is associated with platelet microparticles. *Blood.* 1992;80(9):2246–51.
48. Tati R, Kristoffersson AC, Ståhl AL, Rebetz J, Wang L, Licht C, et al. Complement activation associated with ADAMTS13 deficiency in human and murine thrombotic microangiopathy. *J Immunol.* 2013;191(5):2184–93.
49. Khan I, Zucker-Franklin D, Karpatkin S. Microthrombocytosis and platelet fragmentation associated with idiopathic/autoimmune thrombocytopenic purpura. *Br J Haematol.* 1975;31(4):449–60.
50. Brogan PA, Shah V, Brachet C, Harnden A, Mant D, Klein N, et al. Endothelial and platelet microparticles in vasculitis of the young. *Arthritis Rheum.* 2004;50(3):927–36.
51. Karpman D. Haemolytic uremic syndrome and thrombotic thrombocytopenic purpura. *Curr Paediatr.* 2002;12:569–74.

52. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med*. 2014;371(19):1847–8.
53. Besbas N, Karpman D, Landau D, Loirat C, Proesmans W, Remuzzi G, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. *Kidney Int*. 2006;70(3):423–31.
54. McKee ML, O'Brien AD. Investigation of enterohemorrhagic *Escherichia coli* O157:H7 adherence characteristics and invasion potential reveals a new attachment pattern shared by intestinal *E. coli*. *Infect Immun*. 1995;63(5):2070–4.
55. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet*. 2005;365(9464):1073–86.
56. Proulx F, Seidman EG, Karpman D. Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res*. 2001;50(2):163–71.
57. Zoja C, Buelli S, Morigi M. Shiga toxin-associated hemolytic uremic syndrome: pathophysiology of endothelial dysfunction. *Pediatr Nephrol*. 2010;25(11):2231–40.
58. Karpman D, Ståhl AL. Enterohemorrhagic *Escherichia coli* pathogenesis and the host response. *Microbiol Spectrum*. 2014;2(5):1–15.
59. Tazzari PL, Ricci F, Carnicelli D, Caprioli A, Tozzi AE, Rizzoni G, et al. Flow cytometry detection of Shiga toxins in the blood from children with hemolytic uremic syndrome. *Cytometry B Clin Cytom*. 2004;61(1):40–4.
60. Brigotti M, Tazzari PL, Ravanelli E, Carnicelli D, Rocchi L, Arfilli V, et al. Clinical relevance of Shiga toxin concentrations in the blood of patients with hemolytic uremic syndrome. *Pediatr Infect Dis J*. 2011;30(6):486–90.
61. Uchida H, Kiyokawa N, Horie H, Fujimoto J, Takeda T. The detection of Shiga toxins in the kidney of a patient with hemolytic uremic syndrome. *Pediatr Res*. 1999;45(1):133–7.
62. Chaisri U, Nagata M, Kurazono H, Horie H, Tongtawe P, Hayashi H, et al. Localization of Shiga toxins of enterohaemorrhagic *Escherichia coli* in kidneys of paediatric and geriatric patients with fatal haemolytic uraemic syndrome. *Microb Pathog*. 2001;31(2):59–67.
63. Cooling LL, Walker KE, Gille T, Koerner TA. Shiga toxin binds human platelets via globotriaosylceramide (Pk antigen) and a novel platelet glycosphingolipid. *Infect Immun*. 1998;66(9):4355–66.
64. van Setten PA, Monnens LA, Verstraten RG, van den Heuvel LP, van Hinsbergh VW. Effects of verocytotoxin-1 on nonadherent human monocytes: binding characteristics, protein synthesis, and induction of cytokine release. *Blood*. 1996;88(1):174–83.
65. van de Kar NC, Monnens LA, Karmali MA, van Hinsbergh VW. Tumor necrosis factor and interleukin-1 induce expression of the verocytotoxin receptor globotriaosylceramide on human endothelial cells: implications for the pathogenesis of the hemolytic uremic syndrome. *Blood*. 1992;80(11):2755–64.
66. Brigotti M, Carnicelli D, Arfilli V, Tamassia N, Borsetti F, Fabbri E, et al. Identification of TLR4 as the receptor that recognizes Shiga toxins in human neutrophils. *J Immunol*. 2013;191(9):4748–58.
67. Louise CB, Obrig TG. Specific interaction of *Escherichia coli* O157:H7-derived Shiga-like toxin II with human renal endothelial cells. *J Infect Dis*. 1995;172(5):1397–401.
68. Karpman D, Hakansson A, Perez MT, Isaksson C, Carlemalm E, Caprioli A, et al. Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. *Infect Immun*. 1998;66(2):636–44.
69. Hughes AK, Stricklett PK, Kohan DE. Cytotoxic effect of Shiga toxin-1 on human proximal tubule cells. *Kidney Int*. 1998;54(2):426–37.
70. Tesh VL, Ramegowda B, Samuel JE. Purified Shiga-like toxins induce expression of proinflammatory cytokines from murine peritoneal macrophages. *Infect Immun*. 1994;62(11):5085–94.
71. Guessous F, Marcinkiewicz M, Polanowska-Grabowska R, Keepers TR, Obrig T, Gear AR. Shiga toxin 2 and lipopolysaccharide cause monocytic THP-1 cells to release factors which activate platelet function. *Thromb Haemost*. 2005;94(5):1019–27.

72. Guessous F, Marcinkiewicz M, Polanowska-Grabowska R, Kongkhum S, Heatherly D, Obrig T, et al. Shiga toxin 2 and lipopolysaccharide induce human microvascular endothelial cells to release chemokines and factors that stimulate platelet function. *Infect Immun*. 2005;73(12):8306–16.
73. Karpman D, Papadopoulos D, Nilsson K, Sjögren AC, Mikaelsson C, Lethagen S. Platelet activation by Shiga toxin and circulatory factors as a pathogenetic mechanism in the hemolytic uremic syndrome. *Blood*. 2001;97(10):3100–8.
74. Karpman D, Manea M, Vaziri-Sani F, Ståhl AL, Kristoffersson AC. Platelet activation in hemolytic uremic syndrome. *Semin Thromb Hemost*. 2006;32(2):128–45.
75. Ståhl AL, Svensson M, Morgelin M, Svanborg C, Tarr PI, Mooney JC, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets through TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood*. 2006;108(1):167–76.
76. Bolande RP, Kaplan BS. Experimental studies on the hemolytic-uremic syndrome. *Nephron*. 1985;39(3):228–36.
77. Fong JS, Kaplan BS. Impairment of platelet aggregation in hemolytic uremic syndrome: evidence for platelet “exhaustion”. *Blood*. 1982;60(3):564–70.
78. Sassetti B, Vizcarguenaga MI, Zanaro NL, Silva MV, Kordich L, Florentini L, et al. Hemolytic uremic syndrome in children: platelet aggregation and membrane glycoproteins. *J Pediatr Hematol Oncol*. 1999;21(2):123–8.
79. Appiani AC, Edefonti A, Bettinelli A, Cossu MM, Paracchini ML, Rossi E. The relationship between plasma levels of the factor VIII complex and platelet release products (beta-thromboglobulin and platelet factor 4) in children with the hemolytic-uremic syndrome. *Clin Nephrol*. 1982;17(4):195–9.
80. Tsai HM, Chandler WL, Sarode R, Hoffman R, Jelacic S, Habeeb RL, et al. von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Res*. 2001;49(5):653–9.
81. van de Kar NC, van Hinsbergh VW, Brommer EJ, Monnens LA. The fibrinolytic system in the hemolytic uremic syndrome: in vivo and in vitro studies. *Pediatr Res*. 1994;36(2):257–64.
82. Morigi M, Galbusera M, Binda E, Imberti B, Gastoldi S, Remuzzi A, et al. Verotoxin-1-induced up-regulation of adhesive molecules renders microvascular endothelial cells thrombogenic at high shear stress. *Blood*. 2001;98(6):1828–35.
83. 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.
84. Del Conde I, Cruz MA, Zhang H, Lopez JA, Afshar-Kharghan V. Platelet activation leads to activation and propagation of the complement system. *J Exp Med*. 2005;201(6):871–9.
85. Endresen GK, Mellbye OJ. Studies on the binding of complement factor C3 to the surface of human blood platelets. *Haemostasis*. 1984;14(3):269–80.
86. Sandvik T, Endresen GK, Forre O. Studies on the binding of complement factor C4 in human platelets. Complement activation by means of cold agglutinins. *Int Arch Allergy Appl Immunol*. 1984;74(2):152–7.
87. Nunez D, Charriaut-Marlangue C, Barel M, Benveniste J, Frade R. Activation of human platelets through gp140, the C3d/EBV receptor (CR2). *Eur J Immunol*. 1987;17(4):515–20.
88. Zimmerman TS, Kolb WP. Human platelet-initiated formation and uptake of the C5-9 complex of human complement. *J Clin Invest*. 1976;57(1):203–11.
89. Polley MJ, Nachman RL. Human complement in thrombin-mediated platelet function: uptake of the C5b-9 complex. *J Exp Med*. 1979;150(3):633–45.
90. Polley MJ, Nachman RL. Human platelet activation by C3a and C3a des-arg. *J Exp Med*. 1983;158(2):603–15.
91. Dixon RH, Rosse WF. Mechanism of complement-mediated activation of human blood platelets in vitro: comparison of normal and paroxysmal nocturnal hemoglobinuria platelets. *J Clin Invest*. 1977;59(2):360–8.

92. Nicholson-Weller A, March JP, Rosen CE, Spicer DB, Austen KF. Surface membrane expression by human blood leukocytes and platelets of decay-accelerating factor, a regulatory protein of the complement system. *Blood*. 1985;65(5):1237–44.
93. Tschopp J, Jenne DE, Hertig S, Preissner KT, Morgenstern H, Sapino AP, et al. Human megakaryocytes express clusterin and package it without apolipoprotein A-1 into alpha-granules. *Blood*. 1993;82(1):118–25.
94. Yu GH, Holers VM, Seya T, Ballard L, Atkinson JP. Identification of a third component of complement-binding glycoprotein of human platelets. *J Clin Invest*. 1986;78(2):494–501.
95. Morgan BP. Isolation and characterization of the complement-inhibiting protein CD59 antigen from platelet membranes. *Biochem J*. 1992;282(Pt 2):409–13.
96. Vaziri-Sani F, Hellwage J, Zipfel PF, Sjöholm AG, Iancu R, Karpman D. Factor H binds to washed human platelets. *J Thromb Haemost*. 2005;3(1):154–62.
97. Licht C, Pluthero FG, Li L, Christensen H, Habbig S, Hoppe B, et al. Platelet-associated complement factor H in healthy persons and patients with atypical HUS. *Blood*. 2009;114(20):4538–45.
98. Kenney DM, Davis 3rd AE. Association of alternative complement pathway components with human blood platelets: secretion and localization of factor D and beta 1H Globulin. *Clin Immunol Immunopathol*. 1981;21(3):351–63.
99. Orth D, Khan AB, Naim A, Grif K, Brockmeyer J, Karch H, 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.
100. Nevard CH, Jurd KM, Lane DA, Philippou H, Haycock GB, Hunt BJ. Activation of coagulation and fibrinolysis in childhood diarrhoea-associated hemolytic uraemic syndrome. *Thromb Haemost*. 1997;78(6):1450–5.
101. Van Geet C, Proesmans W, Arnout J, Vermeylen J, Declerck PJ. Activation of both coagulation and fibrinolysis in childhood hemolytic uremic syndrome. *Kidney Int*. 1998;54(4):1324–30.
102. Chandler WL, Jelacic S, Boster DR, Ciol MA, Williams GD, Watkins SL, et al. Prothrombotic coagulation abnormalities preceding the hemolytic-uremic syndrome. *N Engl J Med*. 2002;346(1):23–32.
103. Bergstein JM, Riley M, Bang NU. Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med*. 1992;327(11):755–9.
104. Bitzan M, Richardson S, Huang C, Boyd B, Petric M, Karmali MA. Evidence that verotoxins (Shiga-like toxins) from *Escherichia coli* bind to P blood group antigens of human erythrocytes in vitro. *Infect Immun*. 1994;62(8):3337–47.
105. Louise CB, Obrig TG. Shiga toxin-associated hemolytic-uremic syndrome: combined cytotoxic effects of Shiga toxin, interleukin-1 beta, and tumor necrosis factor alpha on human vascular endothelial cells in vitro. *Infect Immun*. 1991;59(11):4173–9.
106. Morigi M, Galbusera M, Gastoldi S, Locatelli M, Buelli S, Pezzotta A, 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.
107. Monnens L, Molenaar J, Lambert PH, Proesmans W, van Munster P. The complement system in hemolytic-uremic syndrome in childhood. *Clin Nephrol*. 1980;13(4):168–71.
108. Robson WL, Leung AK, Fick GH, McKenna AI. Hypocomplementemia and leukocytosis in diarrhea-associated hemolytic uremic syndrome. *Nephron*. 1992;62(3):296–9.
109. Thurman JM, Mariani R, Emlen W, Wood S, Smith C, Akana H, et al. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol*. 2009;4(12):1920–4.
110. Ge S, Hertel B, Emden SH, Beneke J, Menne J, Haller H, et al. Microparticle generation and leucocyte death in Shiga toxin-mediated HUS. *Nephrol Dial Transplant*. 2012;27(7):2768–75.
111. Loirat C, Noris M, Fremeaux-Bacchi V. Complement and the atypical hemolytic uremic syndrome in children. *Pediatr Nephrol*. 2008;23(11):1957–72.

112. Békássy ZD, Kristoffersson AC, Cronqvist M, Roumenina LT, Rybkine T, Vergoz L, et al. Eculizumab in an anephric patient with atypical haemolytic uraemic syndrome and advanced vascular lesions. *Nephrol Dial Transplant*. 2013;28(11):2899–907.
113. Delvaeye M, Noris M, De Vriese A, Esmon CT, Esmon NL, Ferrell G, et al. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2009;361(4):345–57.
114. Ståhl AL, Kristoffersson A, Olin AI, Olsson ML, Roodhooft AM, Proesmans W, et al. A novel mutation in the complement regulator clusterin in recurrent hemolytic uremic syndrome. *Mol Immunol*. 2009;46(11-12):2236–43.
115. Dragon-Durey MA, Sethi SK, Bagga A, Blanc C, Blouin J, Ranchin B, et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol*. 2010;21(12):2180–7.
116. Manuelian T, Hellwage J, Meri S, Caprioli J, Noris M, Heinen S, et al. Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J Clin Invest*. 2003;111(8):1181–90.
117. Skerka C, Zipfel PF. Complement factor H related proteins in immune diseases. *Vaccine*. 2008;26 Suppl 8:19–14.
118. Roumenina LT, Frimat M, Miller EC, Provot F, Dragon-Durey MA, Bordereau P, et al. A prevalent C3 mutation in aHUS patients causes a direct C3 convertase gain of function. *Blood*. 2012;119(18):4182–91.
119. Roumenina LT, Jablonski M, Hue C, Blouin J, Dimitrov JD, Dragon-Durey MA, et al. Hyperfunctional C3 convertase leads to complement deposition on endothelial cells and contributes to atypical hemolytic uremic syndrome. *Blood*. 2009;114(13):2837–45.
120. Sartz L, Olin AI, Kristoffersson AC, Ståhl AL, Johansson ME, Westman K, et al. A novel C3 mutation causing increased formation of the C3 convertase in familial atypical hemolytic uremic syndrome. *J Immunol*. 2012;188(4):2030–7.
121. Brackman D, Sartz L, Leh S, Kristoffersson AC, Bjerre A, Tati R, et al. Thrombotic microangiopathy mimicking membranoproliferative glomerulonephritis. *Nephrol Dial Transplant*. 2011;26(10):3399–403.
122. Vaziri-Sani F, Holmberg L, Sjöholm AG, Kristoffersson AC, Manea M, Fremeaux-Bacchi V, et al. Phenotypic expression of factor H mutations in patients with atypical hemolytic uremic syndrome. *Kidney Int*. 2006;69(6):981–8.
123. Marinozzi MC, Vergoz L, Rybkine T, Ngo S, Bettoni S, Pashov A, et al. Complement factor B mutations in atypical hemolytic uremic syndrome-disease-relevant or benign? *J Am Soc Nephrol*. 2014;25(9):2053–65.
124. de Jorge EG, Macor P, Paixao-Cavalcante D, Rose KL, Tedesco F, Cook HT, et al. The development of atypical hemolytic uremic syndrome depends on complement C5. *J Am Soc Nephrol*. 2011;22(1):137–45.
125. Frimat M, Tabarin F, Dimitrov JD, Poitou C, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, et al. Complement activation by heme as a secondary hit for atypical hemolytic uremic syndrome. *Blood*. 2013;122(2):282–92.
126. Manea M, Karpman D. Molecular basis of ADAMTS13 dysfunction in thrombotic thrombocytopenic purpura. *Pediatr Nephrol*. 2009;24(3):447–58.
127. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood*. 2002;100(12):4033–9.
128. Noris M, Ruggenenti P, Perna A, Orisio S, Caprioli J, Skerka C, et al. Hypocomplementemia discloses genetic predisposition to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: role of factor H abnormalities. Italian Registry of Familial and Recurrent Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura. *J Am Soc Nephrol*. 1999;10(2):281–93.
129. Reti M, Farkas P, Csuka D, Razso K, Schlammadinger A, Udvardy ML, et al. Complement activation in thrombotic thrombocytopenic purpura. *J Thromb Haemost*. 2012;10(5):791–8.

130. Westwood JP, Langley K, Heelas E, Machin SJ, Scully M. Complement and cytokine response in acute Thrombotic Thrombocytopenic Purpura. *Br J Haematol.* 2014;164(6): 858–66.
131. Mant MJ, Cauchi MN, Medley G. Thrombotic thrombocytopenic purpura: report of a case with possible immune etiology. *Blood.* 1972;40(3):416–21.
132. Weisenburger DD, O'Conner ML, Hart MN. Thrombotic thrombocytopenic purpura with C3 vascular deposits: report of a case. *Am J Clin Pathol.* 1977;67(1):61–3.
133. Chapin J, Weksler B, Magro C, Laurence J. Eculizumab in the treatment of refractory idiopathic thrombotic thrombocytopenic purpura. *Br J Haematol.* 2012;157(6):772–4.
134. Ruiz-Torres MP, Casiraghi F, Galbusera M, Macconi D, Gastoldi S, Todeschini M, et al. Complement activation: the missing link between ADAMTS-13 deficiency and microvascular thrombosis of thrombotic microangiopathies. *Thromb Haemost.* 2005;93(3):443–52.
135. Turner NA, Moake J. Assembly and activation of alternative complement components on endothelial cell-anchored ultra-large von Willebrand factor links complement and hemostasis-thrombosis. *PLoS One.* 2013;8(3), e59372.
136. Feng S, Liang X, Cruz MA, Vu H, Zhou Z, Pemmaraju N, et al. The interaction between factor H and Von Willebrand factor. *PLoS One.* 2013;8(8), e73715.
137. Rayes J, Roumenina LT, Dimitrov JD, Repesse Y, Ing M, Christophe O, et al. The interaction between factor H and VWF increases factor H cofactor activity and regulates VWF pro-thrombotic status. *Blood.* 2014;123(1):121–5.
138. Nolasco L, Nolasco J, Feng S, Afshar-Kharghan V, Moake J. Human complement factor H is a reductase for large soluble von Willebrand factor multimers—brief report. *Arterioscler Thromb Vasc Biol.* 2013;33(11):2524–8.
139. Jimenez JJ, Jy W, Mauro LM, Horstman LL, Ahn YS. Elevated endothelial microparticles in thrombotic thrombocytopenic purpura: findings from brain and renal microvascular cell culture and patients with active disease. *Br J Haematol.* 2001;112(1):81–90.
140. Jimenez JJ, Jy W, Mauro LM, Horstman LL, Soderland C, Ahn YS. Endothelial microparticles released in thrombotic thrombocytopenic purpura express von Willebrand factor and markers of endothelial activation. *Br J Haematol.* 2003;123(5):896–902.
141. Jy W, Jimenez JJ, Mauro LM, Horstman LL, Cheng P, Ahn ER, et al. Endothelial microparticles induce formation of platelet aggregates via a von Willebrand factor/ristocetin dependent pathway, rendering them resistant to dissociation. *J Thromb Haemost.* 2005;3(6): 1301–8.
142. Hunder GG, Arend WP, Bloch DA, Calabrese LH, Fauci AS, Fries JF, et al. The American College of Rheumatology 1990 criteria for the classification of vasculitis. Introduction. *Arthritis Rheum.* 1990;33(8):1065–7.
143. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Maeda N, et al. The role of neutrophils in the induction of glomerulonephritis by anti-myeloperoxidase antibodies. *Am J Pathol.* 2005;167(1):39–45.
144. Abdulahad WH, Stegeman CA, Kallenberg CG. Review article: The role of CD4(+) T cells in ANCA-associated systemic vasculitis. *Nephrology (Carlton).* 2009;14(1):26–32.
145. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, et al. Antineutrophil cytoplasmic auto-antibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest.* 2002;110(7):955–63.
146. Primo VC, Marusic S, Franklin CC, Goldmann WH, Achaval CG, Smith RN, et al. Anti-PR3 immune responses induce segmental and necrotizing glomerulonephritis. *Clin Exp Immunol.* 2010;159(3):327–37.
147. Xing GQ, Chen M, Liu G, Heeringa P, Zhang JJ, Zheng X, et al. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol.* 2009;29(3):282–91.
148. Magro CM, Crowson AN, Harrist TJ. The use of antibody to C5b-9 in the subclassification of lupus erythematosus. *Br J Dermatol.* 1996;134(5):855–62.

149. Vassallo G, Newton RW, Chieng SE, Haeney MR, Shabani A, Arkwright PD. Clinical variability and characteristic autoantibody profile in primary C1q complement deficiency. *Rheumatology*. 2007;46(10):1612–4.
150. Jönsson G, Sjöholm AG, Truedsson L, Bengtsson AA, Braconier JH, Sturfelt G. Rheumatological manifestations, organ damage and autoimmunity in hereditary C2 deficiency. *Rheumatology*. 2007;46(7):1133–9.
151. Schreiber A, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol*. 2009; 20(2):289–98.
152. Huugen D, van Esch A, Xiao H, Peutz-Kootstra CJ, Buurman WA, Tervaert JW, et al. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int*. 2007;71(7):646–54.
153. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol*. 2007;170(1):52–64.
154. Brogan PA, Dillon MJ. Endothelial microparticles and the diagnosis of the vasculitides. *Intern Med*. 2004;43(12):1115–9.
155. Erdbruegger U, Grossheim M, Hertel B, Wyss K, Kirsch T, Woywodt A, et al. Diagnostic role of endothelial microparticles in vasculitis. *Rheumatology (Oxford)*. 2008;47(12):1820–5.
156. Daniel L, Fakhouri F, Joly D, Mouthon L, Nusbaum P, Grunfeld JP, et al. Increase of circulating neutrophil and platelet microparticles during acute vasculitis and hemodialysis. *Kidney Int*. 2006;69(8):1416–23.
157. Hong Y, Eleftheriou D, Hussain AA, Price-Kuehne FE, Savage CO, Jayne D, et al. Anti-neutrophil cytoplasmic antibodies stimulate release of neutrophil microparticles. *J Am Soc Nephrol*. 2012;23(1):49–62.
158. Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. *J Immunol*. 1998;161(8):4382–7.
159. Pitanga TN, de Aragao FL, Rocha VC, Meirelles T, Borges VM, Goncalves MS, et al. Neutrophil-derived microparticles induce myeloperoxidase-mediated damage of vascular endothelial cells. *BMC Cell Biol*. 2014;15:21.
160. Wong CS, Mooney JC, Brandt JR, Staples AO, Jelacic S, Boster DR, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multi-variable analysis. *Clin Infect Dis*. 2012;55(1):33–41.
161. Menne J, Nitschke M, Stingle R, Abu-Tair M, Beneke J, Bramstedt J, et al. Validation of treatment strategies for enterohaemorrhagic *Escherichia coli* O104:H4 induced haemolytic uraemic syndrome: case-control study. *BMJ*. 2012;345, e4565.
162. Karpman D. Management of Shiga toxin-associated *Escherichia coli*-induced haemolytic uraemic syndrome: randomized clinical trials are needed. *Nephrol Dial Transplant*. 2012;27(10):3669–74.
163. Lapeyraque AL, Malina M, Fremaux-Bacchi V, Boppel T, Kirschfink M, Oualha M, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med*. 2011;364(26):2561–3.
164. Loos S, Ahlenstiel T, Kranz B, Staude H, Pape L, Hartel C, et al. An Outbreak of Shiga Toxin-Producing *Escherichia coli* O104:H4 Hemolytic Uremic Syndrome in Germany: Presentation and Short-term Outcome in Children. *Clin Infect Dis*. 2012;55(6):753–9.
165. Kielstein JT. Best supportive care and therapeutic plasma exchange with or without eculizumab in Shiga toxin-producing *E. coli* O104:H4-induced hemolytic uremic syndrome: An analysis of the German STEC-HUS registry. *Nephrol Dial Transplant*. 2012; 27(10):3807–15.
166. Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2013;368(23):2169–81.
167. Zuber J, Le Quintrec M, Krid S, Bertoye C, Gueutin V, Lahoche A, et al. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant*. 2012;12(12):3337–54.

168. Tsai E, Chapin J, Laurence JC, Tsai HM. Use of eculizumab in the treatment of a case of refractory, ADAMTS13-deficient thrombotic thrombocytopenic purpura: additional data and clinical follow-up. *Br J Haematol.* 2013;162(4):558–9.
169. Coppo R, Peruzzi L, Amore A, Martino S, Vergano L, Lastauka I, et al. Dramatic effects of eculizumab in a child with diffuse proliferative lupus nephritis resistant to conventional therapy. *Pediatr Nephrol.* 2015;30(1):167–72.
170. El-Husseini A, Hannan S, Awad A, Jennings S, Cornea V, Sawaya BP. Thrombotic microangiopathy in systemic lupus erythematosus: efficacy of eculizumab. *Am J Kidney Dis.* 2015;65(1):127–30.
171. Ricklin D, Lambris JD. Progress and trends in complement therapeutics. *Adv Exp Med Biol.* 2013;735:1–22.

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