Review

Atypical Hemolytic-Uremic Syndrome
The Interplay Between Complements and the Coagulation System

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Hemolytic-uremic syndrome (HUS) is a rare life-threatening disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, and impaired renal function. A thrombotic microangiopathy underlies the clinical features of HUS. In the majority of cases, HUS follows an infection with toxin-producing bacteria such as verotoxin-producing *Escherichia coli.* In some cases, HUS is not preceded by a clinically apparent infection, and therefore, is named *atypical* HUS. The prognosis of atypical HUS is poor. While mortality approaches 25% during the acute phase, end-stage renal disease develops in nearly half of patients within a year. Evidence is accumulating that complement activation through the alternative pathway is at the heart of the pathophysiology leading to atypical HUS. Genetic abnormalities involving complement regulatory proteins and complement components form the molecular basis for complement activation. Since microvascular thrombosis is a quintessential feature of atypical HUS, complements and the coagulation system must work in tandem to give rise to the pathologic alterations observed in this condition. Here, a brief discussion of clinical and morphologic features of atypical HUS is followed by a concise presentation of the complement and coagulation systems. The interplay between complements and the coagulation system is graphically highlighted. Last but not least, conventional and emerging therapies for atypical HUS are outlined.

INTRODUCTION

Thrombotic microangiopathy occurs in several distinct clinical settings such as hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura, antiphospholipid syndrome, scleroderma renal crisis, and malignant hypertension. Hemolytic-uremic syndrome is characterized by endothelial injury and microvascular thrombosis resulting in microangiopathic hemolytic anemia, thrombocytopenia, and ischemic injury to organs, especially to the kidneys.[1,2] In the majority of patients with HUS, a bacterial infection such as gastroenteritis, often hemorrhagic, due to Shiga toxin-producing *Escherichia coli* precedes thrombotic microangiopathy within a week. In approximately 10% of patients with HUS, a preceding bacterial infection is not identified (known as *atypical* HUS).[1,2] Accumulating evidence indicates an important role for the complement system in the pathogenesis of atypical HUS.[1,2] The reciprocal interactions between complements and the coagulation system might provide the molecular basis for vascular thrombosis observed in atypical HUS.

CLINICAL PRESENTATION

Clinical manifestations of atypical HUS are a consequence of microvascular thrombosis resulting in ischemic injury and microangiopathic hemolysis (Figure 1).[2] Atypical HUS can occur at any age. While usually abrupt in onset, the presentation can be insidious in nearly 20% of patients. A hemoglobin concentration below 10 g/dL, a platelet count below

$150 \times 10^9/L$ (usually between $30 \times 10^9/L$ and $60 \times 10^9/L$), and impaired kidney function are often found on presentation. Laboratory tests also disclose features of intravascular hemolysis including elevated serum lactate dehydrogenase and reduced serum haptoglobin levels. Fragmented erythrocytes (schistocytes) and reticulocytes are seen on blood films. Serum concentration of complement C3 may be reduced. The most frequent manifestations of kidney disease in atypical HUS are azotemia, hypertension, proteinuria, and hematuria. Kidney function is frequently severely impaired necessitating renal replacement therapy. Hypertension could be severe as a result of hyperreninemia and volume expansion. Proteinuria can be in the nephrotic

range. Extrarenal manifestations occur in about 20% of patients. Neurological manifestations occur in approximately 10% of patients and include altered mental status from drowsiness to come, focal neurological deficits, and seizure. Cardiac and distal limb ischemia can occur in some patients. A catastrophic presentation due to the involvement of multiple organs is observed in approximately 5% of patients.

PATHOLOGICAL FEATURES

Histologically, atypical HUS is indistinguishable from HUS caused by toxin-producing bacteria. Although atypical HUS can affect various vascular beds, pathologic features in the kidney have been
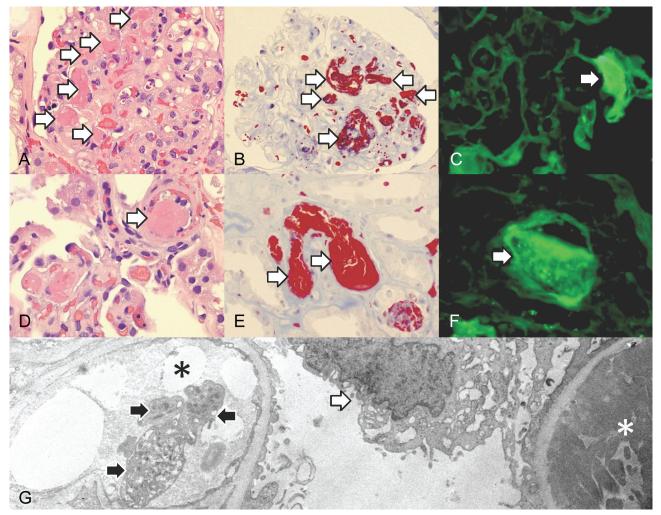

Figure 1. Renal thrombotic microangiopathy in atypical hemolytic-uremic syndrome (Courtesy of Xu Zeng, MD, PhD, Nephrocor Bostwick Laboratory). **A** and **B**, microthrombi (arrows) in glomerular capillaries. **C**, immunofluorescence micrograph revealing a fibrin thrombus (arrow) in a glomerular capillary. **D** and **E**, thrombosis (arrows) of 2 small arteries. F, immunofluorescence micrography revealing a fibrin thrombus (arrow) partially occluding a small artery. **G**, electron micrography demonstrating a thrombosed (white asterisk) glomerular capillary loop. A patent glomerular capillary (black asterisk) contains 3 platelets attached to each other (black arrows). A podocyte (white arrow) is seen in the urinary space. (A and D, hematoxylin-eosin; B and E, Masson's trichrome; and C and F, antiserum against fibrin/fibrinogen).
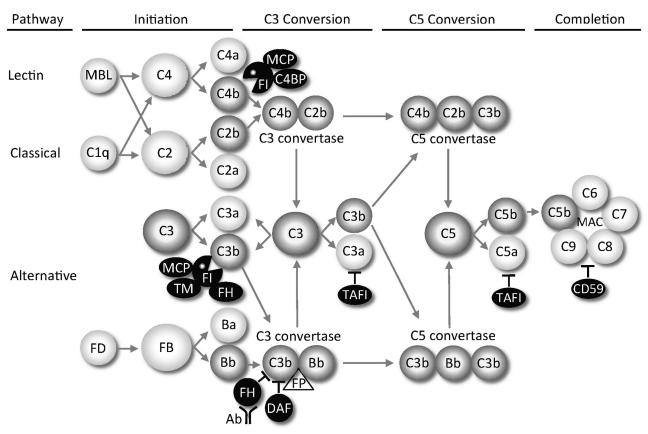
best studied.[3] In the acute phase, there is thickening of glomerular capillary walls and arterioles due to accumulation of plasma proteins including fibrin or fibrinogen in the subendothelial zone. This is in part due to the loss of structural and functional integrity of vascular endothelial cells. Thrombi can be identified in glomerular capillaries, arterioles, or arteries (Figure 1). However, thrombi are not necessary for making a histologic diagnosis of atypical HUS. Endothelial cell swelling or denudation often accompanies thrombosis. Glomerular capillaries and arterioles can undergo fibrinoid necrosis consisting of fibrin, cellular debris, and rare neutrophils. Arcuate and interlobular arteries frequently develop edematous or mucoid intimal expansion resulting in the narrowing of vascular lumen. Immunohistological examination demonstrates irregular deposition of fibrin, immunoglobulin M, C3, and C1q in areas of fibrinoid necrosis and edematous intimal expansion. In the subacute phase, mesangial cell interposition and formation of new basement membrane material results in remodeling of glomerular capillary walls. These changes are reminiscent of glomerular structural alterations observed in membranoproliferative glomerulonephritis, although with less glomerular hypercellularity. Chronic glomerular injury leads to segmental or global glomerular sclerosis. Tubular atrophy and interstitial fibrosis prevail. Collagen deposition in the intima of arterioles and arteries give rise to arteriolosclerosis and arteriosclerosis, respectively. Concentric laminations in the fibrotic intima often result in an "onion skin" pattern of vascular injury.

THE COMPLEMENT SYSTEM
Overview

An evolutionary conserved part of the immune system, complements represent a first-line defense system against invading pathogens.[4] Initially recognized for their complementary bactericidal activity, complements are positioned in the heart of an intricate network of biological systems that regulate innate and adaptive immunity, waste disposal, angiogenesis, regenerative processes, and lipid metabolism. Complements are activated through the classical, lectin and alternative pathways (Figure 2).[4] The classical pathway is strongly activated by immune complexes, which are recognized by the versatile pattern recognition molecule C1q.

Carbohydrates such as those present on microbial surfaces activate the lectin pathway. Following target recognition, proteolytic cleavage of C4 and C2 results in generation of the classical and lectin pathway C3 convertase (C4b2b).[4] The alternative pathway is activated by complex polysaccharides such as those present on the surface of microorganisms. Factor B, factor D, and C3 participate in generation of the alternative pathway C3 convertase (C3bBb), which is stabilized by factor P (also known as properdin). Complement C3 cleavage by the C3 convertases and subsequent C5 cleavage by the C5 convertases results in the formation of C5a and C5b. The latter participates in the assembly of the membrane attack complex (membrane attack complex [MAC], C5b-9, and terminal complement complex). Regardless of origin, all surface-bound C3 convertases can induce activation of the alternative pathway. Therefore, the alternative pathway plays a dominant role in the total complement activity.

Physiologic activities of the complement system include host defense against infections, waste disposal, and connection between innate and adaptive immunity.[4] In the context of an immune response, anaphylatoxins C3a and C5a trigger proinflammatory signaling and attract neutrophils, monocytes, and macrophages to the site of complement activation. Opsonin C3b facilitates phagocytosis, while MAC mediates target cell activation, injury, or lysis in a dose-dependent manner.

Soluble and membrane-bound factors regulate the activity of complements (Figure 2).[5] Soluble complement regulatory proteins include factor I (FI), factor H (FH), and C4-binding protein. Synthetized mainly in the liver, FI is a serine protease that suppresses complement activity by breaking down fluid-phase and cell-bound C3b and C4b. Cofactors are required for the catalytic activity of factor I. A 155-kDa glycoprotein synthesized mainly in the liver, FH serves as a cofactor for FI and facilitates FI-mediated C3b degradation. By removing Bb from C3bBb, FH also accelerates decay of the alternative pathway C3 convertase. Complement C4-binding protein has similar effects on the classical and lectin pathway C3 convertase. Complement regulatory proteins are also found on the surface of most human cells. Membrane cofactor protein (CD46) is a membrane protein expressed by all cells except for erythrocytes. Membrane cofactor protein binds C3b

Figure 2. Complement activation and regulation. The classical, lectin, and alternative pathways of complement activation are demonstrated. The phases of complement activation are also shown. Regulatory proteins are in black circles and ovals. The T sign denotes an inhibitory effect. See text for details. MBL, indicates mannose-binding lectin; MCP, membrane cofactor protein; C4BP, C4-binding protein, FI, factor I; MAC, membrane attack complex; TM, thrombomodulin; FH, factor H; TAFI, thrombin-activatable fibrinolysis inhibitor; FD, factor D; FB, factor B; FP, factor P; DAF, decay accelerating factor; and Ab, antibody.

and C4b and serves as a cofactor for FI. Complement receptor 1 (CR1, CD35, C3b/C4b-receptor) serves as a cofactor for FI and accelerates decay of convertases. Thrombomodulin, a membrane glycoprotein with anticoagulant activity, also facilitates FI-mediated C3b inactivation.

Complement Abnormalities in Atypical Hemolytic-Uremic Syndrome

Reduced C3 and normal C4 levels in the serum of some patients with atypical HUS led to the notion that complements are activated through the alternative pathway. Subsequent work demonstrated that approximately half of patients with atypical HUS have hereditary genetic defects involving soluble and membrane-bound proteins that regulate complement activity (Table).[1,2] Loss of function mutations involving FH, FI, membrane cofactor protein, and thrombomodulin have been associated with atypical HUS. Enhanced

Complement Abnormalities in Atypical Hemolytic-Uremic Syndrome

Complement Abnormality	Prevalence, %	
	Nonfamilial	Familial
Factor H	15 to 20	40 to 50
Anti-factor H antibodies	6 to 10	Not known
Membrane cofactor protein	6 to 10	7 to 15
Complement C3	4 to 6	8 to 10
Factor I	3 to 6	5 to 10
Thrombomodulin	2	9
Factor B	Rare	1 to 2

complement activity could also be the result of increased activity of individual complement components. Of note, increased FB and C3 activities have been associated with atypical HUS. In some patients with atypical HUS, autoantibodies directed against FH are found. Anti-FH antibodies block the binding of factor H to C3b resulting in unopposed assembly of C3bBb and complement activation through the alternative pathway.

Interplay Between Complements and Coagulation System

Unleashed activation of complements in atypical HUS culminates in intravascular coagulation. Although an association between inflammation and a hypercoagulable state has long been recognized, the interplay between complements and the coagulation system has recently come to light (Figure 3).[6,7] It has been shown that complement activation promotes intravascular coagulation. In return, coagulation reactions can stimulate complement activation. A potent trigger of inflammation, anaphylatoxin C5a induces expression of tissue factor (TF) on endothelial cells, monocytes, and neutrophils (*A* in Figure 3). A membrane protein, TF is the receptor and cofactor for coagulation factor VII and its activated form VIIa.[8] Factor VIIa-TF complex triggers activation of coagulation factors X and IX (*B* in Figure 3). On the surface of the TF-bearing cells, activated factor X (Xa) assembles with activated factor V (Va) to form the prothrombinase complex (Va-Xa).

This activates coagulation factor II (prothrombin) to generate a small amount of thrombin (IIa) (*C* in Figure 3). A multifunctional serine protease, thrombin plays a pivotal role in coagulation and cellular activation.[8] A potent platelet agonist, thrombin binds to protease-activated receptors and induces platelet activation, adhesion, and aggregation (*D* in Figure 3). In addition, thrombin triggers exocytosis of platelet α granules containing coagulation factors I, V, and VIII (*E* in Figure 3). It also activates several coagulation factors, including factors V, VIII, XI, XIII, and plasminogen. Assembly of activated factor IX (IXa) with activated factor VIII (VIIIa) on the surface of platelets causes activation of factor X (Xa; *F* in Figure 3). The prothrombinase complex (Va-Xa) formed on the surface of platelets converts prothrombin to thrombin (*G* in Figure 3). Thrombin cleaves fibrinogen to fibrin and induces activation of coagulation factor XIII, which cross-links and stabilizes fibrin (*H* and *I* in Figure 3).

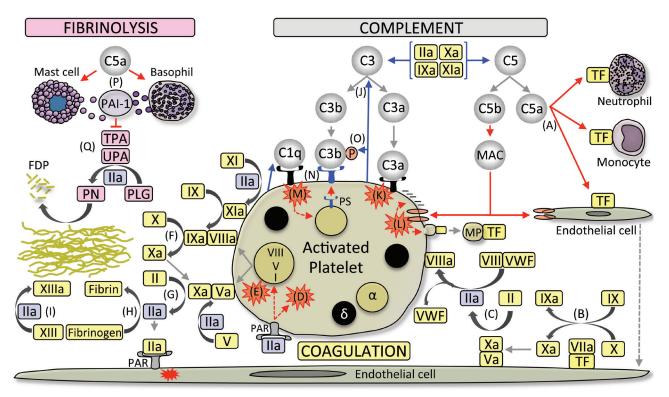Reciprocal interactions between platelets and complements are of particular interest in the

Figure 3. Interplay between complements and the coagulation system. Complement components are labeled by Arabic numerals in grey circles. Coagulation factors are indicated in yellow rectangles except for thrombin, which is in a purple rectangle. A lower case *a* following Roman numerals stands for activated. An inhibitory effect is indicated by an inverted T sign. A red star indicates an activating effect. See text for details. PAI-1 indicates plasminogen activator inhibitor-1; TF, tissue factor; TPA, tissue plasminogen activator; UPA, urokinase plasminogen activator; FDP, fibrin degradation products; PLG, plasminogen; PN, plasmin; PS, P-selectin; MP, microparticle; VWF, Von Willebrand factor; α, platelet alpha granule; δ, platelet delta granule; and PAR, protease-activated receptor.

pathogenesis of atypical HUS.[6,7] Platelets can cleave C3 into its active components C3a and C3b (*J* in Figure 3). Binding of C3a to its receptor on the surface of platelets stimulates activation, adhesion, and aggregation of platelets (*K* in Figure 3). C5b participates in the assembly of MAC on the surface of platelets resulting in generation of negatively charged prothrombotic phospholipids in the cell membrane (*L* in Figure 3). Membrane attack complex also triggers secretion of platelet storage granules and release of the TF-bearing microparticles (*L* in Figure 3). Binding of C1q to its receptor on the surface of platelets stimulates expression of P-selectin and integrins such as GPIIb-IIIa (*M* in Figure 3). A docking site for C3b, P-selectin can facilitate formation of C5 convertase of the alternative pathway of complement activation (*N* in Figure 3). Platelets also stabilize C3b through phosphorylation (*O* in Figure 3). Complement C5a stimulates production of plasminogen activator inhibitor 1 in mast cells and basophils (*P* in Figure 3). A potent inhibitor of tissue plasminogen activator and urokinase plasminogen activator, plasminogen activator inhibitor 1 suppresses generation of plasmin and breakdown of fibrin (*Q* in Figure 3).

PROGNOSIS AND TREATMENT

The prognosis of atypical HUS is poor. While mortality approaches 25% during the acute phase, end-stage renal disease develops in nearly half of patients within a year.[1,2] The outcome of kidney transplantation in patients with atypical HUS is disappointing.[9] Depending on the underlying genetic abnormality, the recurrence rate in renal allograft could be as high as 80%. In the vast majority of cases, recurrent atypical HUS leads to graft loss.

Plasma therapy including plasma exchange and infusion has remained the standard treatment for atypical HUS.[1,2] While plasma infusion replenishes deficient regulatory proteins, plasma exchange therapy has the additional benefit of removing factors that inhibit regulatory proteins. However, in some cases prolonged treatment may be needed for the induction of remission. In addition, plasma therapy may even fail to induce remission. Considering the pivotal role of complements in the pathogenesis of atypical HUS, strategies to directly suppress complement activity represent a logical therapeutic approach. A neutralizing monoclonal antibody directed against complement

C5, eculizumab has been shown to exert salutary effects in patients with atypical HUS.[1,2] Eculizumab blocks cleavage of C5 into C5a and C5b and prevents formation of MAC resulting in attenuation of complement-mediated inflammatory reactions and tissue injury. It is conceivable that beneficial effects of eculizumab in atypical HUS may also be related to reduced activity of the coagulation system. That is, reduced expression of tissue factor on endothelium and immune cells, suppression of the activity of platelets, immune cells, and endothelium, as well as enhanced fibrinolytic activity.

CONFLICT OF INTEREST

None declared.

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