Pathogenesis of Atypical Hemolytic Uremic Syndrome

Yoko Yoshida[1], Hideki Kato[2], Yoichiro Ikeda[1] and Masaomi Nangaku[1]

[1] Division of Nephrology and Endocrinology, the University of Tokyo Hospital, Tokyo, Japan
[2] Department of Prevention of Diabetes and Lifestyle-Related Diseases Graduate School of Medicine, the University of Tokyo, Tokyo, Japan

Atypical hemolytic uremic syndrome (aHUS) is a type of thrombotic microangiopathy (TMA) defined by thrombocytopenia, microangiopathic hemolytic anemia, and renal failure. aHUS is caused by uncontrolled complement activation in the alternative pathway (AP). A variety of genetic defects in complement-related factors or acquired autoantibodies to the complement regulators have been found in 50 to 60% of all cases. Recently, however, the classification and diagnosis of aHUS are becoming more complicated. One reason for this is that some factors, which have not been regarded as complement-related factors, have been reported as predisposing factors for phenotypic aHUS. Given that genotype is highly correlated with the phenotype of aHUS, careful analysis of underlying genetic abnormalities or acquired factors is needed to predict the prognosis or to decide an optimal treatment for the disease. Another reason is that complement dysregulation in the AP have also been found in a part of other types of TMA such as transplantation-related TMA and pregnancy-related complication. Based on these findings, it is now time to redefine aHUS according to the genetic or acquired background of abnormalities.
Here, we review the pathogeneses and the corresponding phenotypes of aHUS and complement-related TMAs.

Key words: Atypical hemolytic uremic syndrome, Complement, Alternative pathway

Introduction

Thrombotic microangiopathy (TMA) is a pathological condition caused by the formation of microvascular thrombi, leading to thrombocytopenia, microangiopathic hemolytic anemia, and organ damage. Various hereditary or acquired etiologies are associated with TMA. The most common forms of TMAs are thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) caused by the infection of Shiga-toxin producing Escherichia coli (STEC), named STEC-HUS. TTP arises from severe deficiency of a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13 (ADAMTS13)[1-3], which is a specific cleaving protease of von Willebrand factor (VWF). Uncleaved VWF multimers due to the lack of ADAMTS13 activity promote thrombi formation in small vessels. STEC-HUS is the most frequent form of HUS, and predominantly found in children. Shiga-toxin binds globotri-aosylceramide on target cell surface and leads to cytotoxicity including protein synthesis and apoptosis, and also induces the secretion of unusually large VWF multimers from endothelial cells[4].

Historically, the term "atypical HUS (aHUS)" was used to describe HUS not caused by the infection of STEC. Therefore, a category of aHUS included not only complement-related TMA, but also TMAs caused by various factors, such as drug, malignancy, pregnancy, transplantation, etc. However, since 1980s, various clinical and experimental studies have shown that 50% to 60% cases of aHUS arise from inherited and/or acquired complement abnormalities in the alternative pathway (AP). According to these progresses, the term "aHUS" came to be used to only describe complement-mediated aHUS and was distinguished from TMAs with coexisting disease or triggers, which were named "secondary TMA" in the criteria of Japan[5, 6]. The classification of aHUS and secondary TMA, however, remains controversial. More
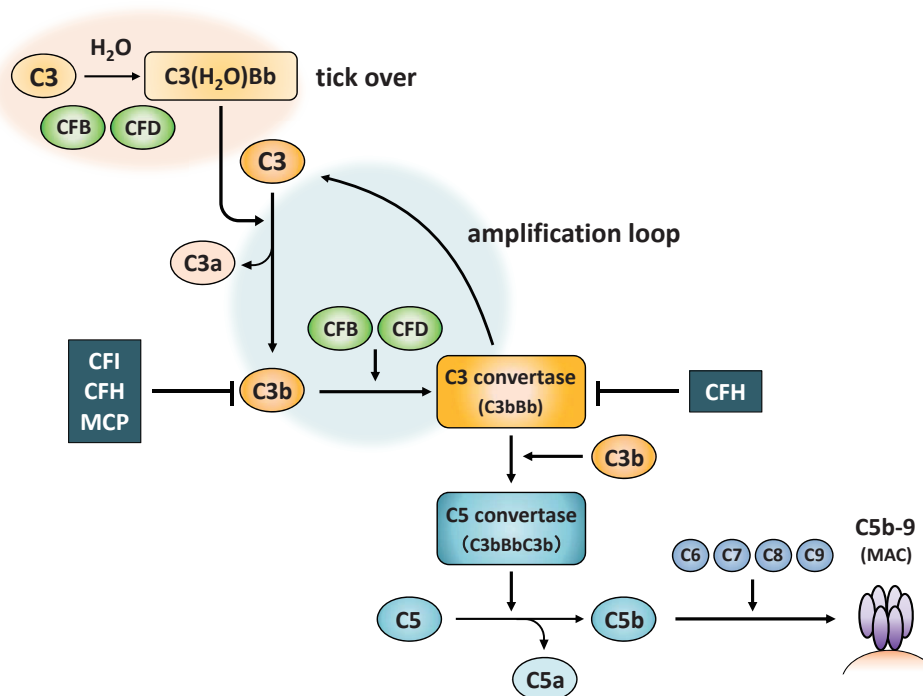
Fig. 1. Alternative pathway of complement

CFB: complement factor B, CFD: complement factor D, CFI: complement factor I, CFH: complement factor H, MCP: membrane cofactor protein, MAC: membrane attack complex

recently, KDIGO controversies conference report has stated that the term "primary aHUS" was preferentially used instead of "aHUS"[7]. Moreover, in this conference report, "secondary TMAs" were re-included into the categories of aHUS, and the use of etiology-based terminology (e.g. pregnancy-aHUS, drug-aHUS) was introduced. To avoid confusion in clinical practice, consistency in the terminology is needed. In this article, the term "aHUS" is used to describe complement-related aHUS, and "secondary TMA" to indicate TMA caused by various underlying diseases or factors.

The Complement System

The complement system is an essential part of innate immunity, and is activated via three pathways: classical pathway, lectin pathway, and AP. Activation any one of these pathways eventually leads to the opsonization of pathogens, and the generation of anaphylatoxin and membrane attack complex (MAC).

Uncontrolled complement activation in the AP is strongly linked with the pathogenesis of aHUS. The activation of both classical and lectin pathway needs specific initiator, but the AP is activated spontaneously at a low level by the hydrolysis of complement factor C3[8, 9] (**Fig. 1**). C3, the most abundant complement protein in plasma, plays an important role in complement activation. The internal thioester bond in C3 is easily hydrolyzed by H_2O, leading to the formation of $C3(H_2O)$. The interaction of $C3(H_2O)$ with complement factor B (CFB) followed by cleavage by complement factor D generates $C3(H_2O)Bb$. This reaction occurs constantly and is called "tick-over". The molecule of $C3(H_2O)Bb$ works as an initial fluid phase C3 convertase, which cleaves C3 into C3a and C3b. The C3b fragment can bind covalently to any surface via its thioester, and forms C3 convertase (C3bBb) in the presence of CFB and CFD. This reaction creates a feedforward loop that cleavages more C3 into C3a and C3b ("C3 amplification"). Furthermore, C3 convertase can recruit another C3b to form C3bBbC3b (C5 convertase) and generate C5a and C5b through C5 cleavage. The C5b molecule binds to C6, C7, C8 and C9 to create MAC (C5b-9), which cause a direct lysis of target by the formation of membrane pores. The anaphylatoxins, C3a and C5a have a strong chemotactic influence on phagocytes, and also cause an increase in vascular permeability via releasing histamine[10].

Generally, $C3(H_2O)Bb$ or C3b is inactivated rapidly by various complement regulatory factors both in fluid phase and on host cell surface. On the other

Table 1. Summary of the genetic or acquired complement abnormalities associated with aHUS

Genetic or acquired abnormalities		Frequency (%)	Main effect of mutant or acquired antibody
Complement regulatory factors	CFH	20%-30%	· Impaired CFH binding to C3b and/or glycosaminoglycans on host cell surface · Reduced cofactor activity*
	CFH/CFHR hybrid	-	· Impaired CFH binding to C3b and/or glycosaminoglycans on host cell surface · Competitive interaction with CFH
	CFI	4%-8%	· Impaired CFI secretion · Reduced proteolytic activity
	MCP	8%-10%	· Reduction in MCP expression · Reduced C3b binding and cofactor activity
	anti-CFH antibody	5%-20%	Inhibition of the complement regulatory function of CFH
Complement activation factors	C3	4%-8%	· Resistance to CFI-mediated inactivation of C3b · Formation of hyperactive C3 convertase
	CFB	<1%-4%	· Resistance of convertase to decay by CFH · Formation of hyperactive C3 convertase
Coagulation-related factors	THBD	3%-5%	· Reduced cofactor activity · Diminished activation of TAFI
	DGKE	8%	Upregulation of prothrombotic factors and platelet activation**
	PLG	-	Reduced fibrinolytic activity**
Other	INF2	-	- **

CFH: complement factor H, CFHR: complement factor H related, CFI: complement factor I, MCP: membrane cofactor protein, C3: complement component C3, CFB: complement factor B, THBD: thrombomodulin, DGKE: diacylglycerol kinase epsilon, PLG: plasminogen, INF2: inverted formin 2, TAFI: thrombin-activatable fibrinolysis inhibitor (plasma procarboxypeptidase B)
*Some predisposing variants reside in N-terminal region of CFH show impaired cofactor activity for CFI.
**Pathologic mechanism of aHUS caused by DGKE, PLG, INF2 variants has not been well described.

hand, once C3b binds to pathogens, which lack a complement regulatory protein, bound C3b preferentially reacts with CFB and CFD and causes the disruption of cell via the formation of MAC. In this manner, the complement attack occurs selectively on foreign substances, but not on the host cells.

Complement Dysregulation in the Patients with aHUS

In the AP, complement factor I (CFI) is a main protease for C3b inactivation. This regulatory process needs specific cofactor like complement factor H (CFH) and membrane cofactor protein (MCP). In the patients with aHUS, uncontrolled complement activation is caused by the loss of function variants in complement regulatory proteins or gain-of-function variants in complement activation factors. We here describe the detailed functions of complement-related factors

and their abnormalities associated with aHUS (**Table 1**).

Genetic Defect of Complement Regulatory Factors
Complement Factor H
CFH is a major complement regulatory factor in the AP, and consists of 20 short consensus proteins (SCRs), each of which has 60 amino residues. CFH functions not only as a cofactor for CFI converting C3b to inactivation form, but also as a decay-accelerating factor via competing with CFB in binding to C3b. This regulatory function depends on the N-terminal region of CFH (SCRs 1-4) containing C3b-binding site[11]. By contrast, C-terminal region (SCRs 19-20) contains both C3b[12, 13] and surface glycan-binding site[14, 15]. Accordingly, CFH SCRs 19-20 are capable of binding to host surface like endothelial cells via glycosaminoglycans like heparin[14] or sialic acid[15] and can exert complement regulatory effect[16].

Predisposing variants in *CFH* are the most fre-

quent abnormalities in aHUS as they account for 20% to 30% of cases[17-20]. On the other hand, the frequency of genetic abnormalities in *CFH* is estimated to be less in Japan compared to that in Western countries and the US, at about 10%[21, 22]. The variants in *CFH* associated with aHUS are mostly heterozygous, are located in SCRs19-20[18, 19], and seem to affect the protein function, but not a quantitative deficiency. Several studies have shown that the pathogenicity of these variants is due to impaired CFH binding to C3b, or heparin or sialic acid[23, 24] expressed on host cell surface, leading to increased C3b and C5b-9 deposition onto host cells.

Complement Factor H Related (CFHR) Protein

The genes encoding the five CFHR (CFHR1-5) proteins reside in close proximity to CFH on chromosome 1q32. Each CFHR proteins have four to nine SCRs, which are high sequence identity to C-terminal region of CFH. Functionally, CFHR3, CFHR4, and CFHR5 proteins show the cofactor activity[25, 26], whereas CFHR1 and CFHR2 proteins are likely to inhibit the formation of C5 convertase[27], C3 convertase[28], respectively. However, these complement regulatory effect are mostly observed at non-physiological concentrations. In addition to these findings, enhanced complement activation via CFHR proteins was also reported; CFHR1 and CFHR5 protein may compete with CFH for binding to bacterial ligands and C3b[29, 30].

Although physiological functions of CFHR proteins remain incompletely characterized, the genetic deletions of these proteins are associated with aHUS. Due to an extremely high sequence identity in the *CFH* and *CFHR* gene family, various non-allele homologous recombinations can occur[31]. The homozygous deletion of *CFHR1* is frequently found in the patients with anti-CFH antibodies as described in the following section. Hybrid protein consisting of CFH and CFHR were also predisposed to aHUS. For instance, a hybrid gene consisting of SCRs1-18 of CFH and SCRs4-5 of CFHR1 encodes a protein identical to S1191L/V1197A CFH mutant protein[32]. These changes cause the impaired control of complement activation on host cell surfaces due to the lack of CFH binding to C3b[33]. Another hybrid protein having SCRs1-4 of CFHR1 and SCRs19-20 of CFH competes with CFH for surface C3b binding[34]. So far, six different patterns of hybrid have been reported in aHUS[31, 35, 36].

Complement Factor I

Serine protease of CFI works as a critical regulator of complement activation that cleaves C3b in the presence of specific cofactor like CFH and MCP. Generally, predisposing variants described in aHUS are heterozygous, and the frequency is reported to be 4% to 8%[17, 20, 37], but no patients have been identified in Japan until now[21, 22]. The majority of variants are located in the exons, which encode the serine protease domain[20]. Predisposing variants result in impaired secretion of CFI or decreased proteolytic activity both in fluid phase and/or on cell surfaces[38, 39].

Membrane Cofactor Protein (CD46)

MCP is a widely expressed transmembrane glycoprotein, and a cofactor for CFI-mediated cleavage of C3b on the cell surface. The extracellular N-terminal domains consist of four SCRs, which are responsible for C3b binding. The frequency of *MCP* variants in aHUS is reported to be 8% to 10%[17-19], and 5% in Japan[22]. Most of predisposing variants related to aHUS are heterozygous and clustered in four extracellular SCRs region[40]. Generally, these variants reduce *MCP* expression, whereas some of them result in functional defect such as reduced C3b binding capacity and cofactor activity[40].

Genetic Defect of Complement Activation Factors
Complement Component C3

C3 is a pivotal component in complement system and mainly synthesized by the liver. *C3* variants have been detected in 4% to 8% patients with aHUS[17, 18], but its frequency in Japan was much higher (31%)[21, 22]. The majority of predisposing variants is heterozygous and clusters on the surface predominantly around CFH binding site[41]. The functional analyses of C3 variants have shown that mutant C3 reduced the binding affinity for CFH and/or MCP, resulted in impaired CFI-mediated inactivation of C3b[41-43]. Moreover, one variant in C3 p.R139W has been reported to increase the binding affinity of C3 for CFB, leading to a hyperactive C3 convertase formation[44].

In Japan, 32 of 104 patients with aHUS had predisposing variants in *C3*[22] and 24 of 32 patients belonging to 16 families had the same p.I1157T variant. This was located in the thioester containing domain of C3, and was likely to be resistant to inactivation by CFI in the presence of MCP[43]. A geographical distribution of the patients carrying this variant was found in a restricted area (Kansai district), especially in Mie[21, 45]. A prevalent C3 variant of p.R161W has also been reported in France[44] and Nethrland[46], the frequency of this variant was 42% (14 of 33 cases) and 78% (11 of 14 cases) of aHUS patients with *C3* variants, respectively[41].

Complement Factor B

CFB is a zymogen that carries the catalytic site for C3 convertase. *CFB* variants in aHUS are rare, accounting for only <1%–4%[20, 47]. Pathogenic variants of *CFB* found in aHUS facilitate the formation of either hyperactive C3 convertase or convertase resistant to inactivation by complement regulatory factors[47, 48]. Patients carrying *CFB* variants generally show decreased C3 levels due to permanent activation of the AP. Marinozzi MC, *et al.* have performed detailed structural and functional analysis of *CFB* variants and suggested that 9 of 15 variants had no correlation with aHUS[48]. This finding suggests that the importance of the functional assessment of identified variants in aHUS patients.

Acquired Abnormalities Related to aHUS

Acquired autoantibodies against CFH have been identified in 5%–20% of patients with aHUS[18, 49, 50] and 19% in Japan[22]. Interestingly, extremely high frequency of antibody-positive patients, an incidence rate of 56%, was reported in India[51]. Children aged 5 to 10 years old can be predominantly affected, but CFH antibodies are also found in adult[52]. Antibodies generally recognize the C-terminal region of CFH, and are demonstrated to inhibit the complement regulatory function of CFH on cell surface[53-56].

Antibodies production against CFH is highly associated with the genetic deletion of CFHR1 protein[49, 54, 57]. Deletions are generally homozygous and sometimes accompanied with the genetic deletion of *CFHR3* or *CFHR4*[49, 54]. Of note, genetic deletion of *CFHR1* is also found in healthy individuals, and its frequencies vary by the ethnic origin[57-61]. Therefore, it remains unclear how homozygous deletion of *CFHR1* gene leads to anti-CFH antibodies production. Bhattacharjee A, *et al.*[62] have proposed one hypothesis to this question. They have showed that CFH has autoantigenic epitope, which can be expressed when microbial molecules bind close to this area. CFHR1 protein has a structurally similar to autoangtigenic conformation of CFH and might function as an immune tolerance. Therefore, the loss of immune tolerance due to the absence of CFHR1 may attribute to autoimmune type of aHUS.

Genetic Variants Unrelated to the Complement System in aHUS

Various factors not associated with complement system have been reported to lead the phenotypic aHUS. We here describe three coagulation-related proteins and one protein associated with nephrotic syndrome as the predisposing factors for aHUS.

Patients with aHUS having variants of thrombomodulin (*THBD*) were firstly described in 2009[63]. Thrombomodulin is anticoagulant glycoprotein, but it also facilitates CFI-mediated C3b inactivation in the presence of cofactor like CFH, and enhances thrombin mediated activation of plasma procarboxypeptidase B (TAFI), which inactivates complement anaphylatoxins C3a and C5a[63]. The frequency of *THBD* variants in aHUS is estimated to be 3%–5%[18, 63]. Generally, predisposing variants are heterozygous and cluster in the lectin-like domain or the serine-threonine rich region[63]. These variants seem to induce excess activation of the AP by reduced CFI-mediated conversion of C3b into inactivated C3b and the activation of TAFI[63]. It is debatable whether thrombomodulin-associated aHUS can be classified as complement-associated.

Diacylglycerol kinase epsilon (DGKE), a lipid kinase family protein, shows anticoagulant effect via diacylglycerols-mediated activation of protein kinase C. In 2013, Lemaire, *et al.* have identified that the recessive *DGKE* variants in 13 aHUS patients belonging to 9 unrelated families[64]. Predisposing variants are generally homozygous or compound heterozygous, and sometimes reside in intronic region[65]. Although the data regarding the frequency was still limited, Schaefer F, *et al.* have reported that *DGKE* variants were identified in 8% of the 101 patients tested[66]. Underlying pathophysiologic mechanism of DGKE-associated aHUS is still unclear, but one possibility is that the loss of function of DGKE results in upregulation of prothrombotic factors and platelet activation. Although some aHUS patients showed the low levels of C3[67, 68], the relevance to complement system is still controversial[69].

Plasminogen (PLG) is the inactivate precursor of plasmin and can degrade fibrin clot. One published paper has identified four genetic variants in the gene of *PLG* in four patients with aHUS[70], and three of four variants were known as plasminogen deficiency-related variants[71]. Therefore, the authors have suggested that reduced fibrinolytic activity compromised the degradation of thrombi in aHUS. Hyvarinen S, *et al.* have reported that plasminogen was not likely to inhibit complement activation both on erythrocytes and endothelial cells, but it hindered platelet aggregation[72].

More recently, Challis, *et al.* have performed whole-exome sequencing of patients with aHUS, who not responsive to anti-complement drug eculizumab, and identified the two variants in the gene of inverted formin 2 (*INF2*) in four patients belonging to two families[73]. INF2 is a formin protein that has actin polymerization and depolymerization activity, and the

variants in this protein are known to cause familial autosomal dominant nephrotic syndrome[74]. Two missense variants of *INF2* detected in aHUS resided in the diaphanous inhibitory domain, which was a mutational hot spot for focal segmental glomerulosclerosis. No efficacy of eculizumab treatment was reported so far, therefore the pathogenesis of INF2-mediated aHUS may be independent of complement activation.

Influence of Each Complement Abnormality on Clinical Characteristics and Prognosis of aHUS

The clinical manifestation and prognosis of aHUS are sometimes affected by each abnormality in aHUS-causing factors. Scaefer F, *et al.* have recently reported the phenotype and genotype correlation of 851 patients in the Grobal aHUS Registry, prior to eculizumab treatment[66]. The investigators have shown that the age at onset of initial aHUS was significantly affected by variants in *MCP* or *CFI*. Patients with *CFH* variants showed increased risk of end-stage renal disease (ESRD), whereas the patients with *MCP* variants were linked with longer ESRD-free survival, which has also been confirmed by previous reports[18, 19, 37]. Although the reports are limited, patients carrying *DGKE* variants generally presented aHUS with multiple relapsing episodes and proteinuria before the age of first year[64, 65, 67, 75].

We have recently performed the epidemiological and genetic studies of Japanese patients with aHUS, and identified the following results[22]. The renal survival rate of the patients carrying *MCP* variant or anti-CFH antibodies was much better compared to *CFH*, *C3*, and the unidentified group. The risk of ESRD in the antibody-positive patients is likely to be significantly lower compared to previous report[52], although the reason for this observation is still unclear. Another finding is that the different characteristics between the patients with C3 p.I1157T, a prevalent variant in Japan, and with other *C3* variants. C3 p.I1157T were associated with high recurrence of aHUS compared to other *C3* variants, but showed better renal outcomes both in the acute phase and during long-time follow-up. Furthermore, most of patients carrying C3 p. I1157T achieved remission with only supportive care or plasma therapy. These results have suggested that clinical presentation might be influenced differently according to individual variants even if the kind of defective protein is the same.

Underlying Complement Activation in Other Types of TMA

In recent years, several research groups have suggested that the genetic or acquired complement abnormalities in the AP were also related to the pathogenesis of secondary TMAs. Jodele S, *et al.*[76] have analyzed six pediatric patients with hematopoietic stem-cell transplant (HSCT)-TMA, and revealed that five of six patients had the deletions in *CFHR3* and *CFHR1* gene, and three of six patients had anti-CFH antibodies. Another study performed by the same group revealed that an elevated soluble C5b-9 (sC5b-9), markers of terminal complement activation in the fluid phase, may be a good predictor of disease prognosis in the patients with HSCT-TMA[77]. The patients with HSCT-TMA presenting proteinuria and increased levels of sC5b-9 at the time of TMA diagnosis showed very poor survival ($<20\%$ at 1 year), whereas HSCT-TMA patients having no proteinuria with normal serum C5b-9 had a survival of 100%.

Pregnancy can be a strong trigger for TMA. A retrospective study of 100 female patients with aHUS have shown that 21 (21%) patients developed aHUS related to pregnancy, and the onset of most cases were found in the postpartum period[78]. Therefore, the development of TMA during pregnancy would be one of important factors to suspect aHUS. HELLP (hemolysis, elevated liver enzyme, and low platelets) syndrome is one severe complication during pregnancy, and can be also classified into TMA. In 2008, Fakhouri F, *et al.* have identified the four variants in *CFH*, *CFI*, and *MCP* in 4 of 11 patients (36%) with HELLP syndrome[79]. In the subsequent larger study of 33 patients, three variants (one in *MCP*, two in *CFI*) have been found in three patients, but one of *CFI* variants had less evidence for uncontrolled complement activation[80]. More recently, Vaught AJ, *et al.*[81] have performed the functional and genetic analysis of 13 HELLP syndrome patients and revealed that these patients showed significantly increased complement activation of AP compared with controls (62% vs 16%, $p=0.009$). Furthermore, 46% of patients with HELLP syndrome had rare germline variants (minor allele frequency <0.01) in the genes of *CFHR1*, *CFHR5*, *C3*, or homozygous genetic deletion of *CFHRs*.

The KDIGO controversies conference report has stated that the patients with pregnancy-associated or *de novo* transplantation associated TMA (aHUS) should have a full complement evaluation due to the high prevalence of rare genetic variants or autoantibodies described in these subgroups[7]. Although the number of reports is still limited, the genetic defects and/or complement activation in the AP have also been found in the secondary TMA patients related to hypertension, IgA nephropathy, or systemic lupus erythematosus, etc. Larger cohort study is needed to clarify the association between complement dysregulation

and the pathogenesis of these secondary TMAs, but it is likely that this kind of coincidence should be extremely rare.

Detection of Complement Abnormalities to Diagnose aHUS

Atypical HUS is clinically diagnosed by exclusion of TTP, STEC-HUS, and secondary TMA. Unfortunately, there are no specific biomarkers to confirm the clinical diagnosis of aHUS. Differentiating aHUS from secondary TMA is sometimes most difficult because no biomarkers can distinguish them.

When aHUS is clinically suspected, a variety of specific protein-based assay are recommended as follows[82]; quantification of complement components and its regulators (C3, C4, CFH, CFI, MCP and CFB), the markers for complement activity (CH50 and AH50), and screening of anti-CFH autoantibodies. Decreased C3 levels, but not C4 may reflect the excess activation of the AP. However, even if these factors are within normal limits, the diagnosis of aHUS is not still ruled out. Genetic screening of genes known to cause aHUS (*CFH*, *CFI*, *MCP*, *C3*, *CFB*, *THBD*, *DGKE*, and *CFHR*) is important to confirm the clinical diagnosis of aHUS and to decide the treatment strategy as well as to estimate the outcome and prognosis. The drawback of genetic analysis is that it takes at least several weeks to obtain the result, and the known genetic abnormalities account for about 50% to 60% of aHUS cases.

Various biomarkers have been studied to reveal the underlying complement activation in aHUS. Increased levels of C5a and sC5b-9 have been detected in the acute phase of aHUS, but these markers do not clearly distinguish aHUS from other types of TMA[46, 83, 84]. Modified HAM test by using GPI-anchored protein-deficient cells showed high specificity in differentiating aHUS from TTP[85]. The hemolytic assay by using non-sensitized sheep red blood cells can be assessed cell-protected function of CFH[21, 86, 87]. It can be useful for rapid detection of *CFH* predisposing variants or anti-CFH autoantibodies, leading to defect C-terminal function of CFH. In addition to CFH-related variants, we have previously reported that C3 p. K1105Q positioned at the CFH binding interface also showed marked hemolysis in the hemolytic assay[21].

Current Therapy for aHUS

A first-line treatment for aHUS had been empirically considered to be plasma treatment (plasma infusion and plasma exchange). This supplies functional complement regulatory factors and/or can remove the abnormal complement-related factors like mutant proteins or anti-CFH antibodies. Although the introduction of plasma therapy has decreased the mortality of patients with aHUS, 48% of pediatric and 67% of adult cases died or reached ESRD within 3-year follow-up[18].

The treatment using complement-inhibiting drug, eculizumab, is now replacing plasma therapy as the gold-standard in the management of aHUS. Eculizumab, a monoclonal humanized antibody against C5, blocks the cleavage of C5 leading to prevent MAC formation, whereas an opsonization by C3b is unaffected. The efficacy and safety of this drug have been described in many reports[88-91]. In pediatric cases, eculizumab administration is recommended as a first-line therapy because children have a high risk of catheter-related complications and a low risk of secondary TMAs[92]. On the other hand, plasma therapy may be the first choice when eculizumab is unavailable or in adult cases having the possibility of secondary TMAs without the involvement of complement abnormalities. In autoimmune type of aHUS, combined therapy of concomitant immunosuppression and plasma therapy may allow better outcomes[51, 52, 92], though some reports have recently implicated that eculizumab treatment might be effective in autoantibody-mediated aHUS. It is still to be elucidated what is the optimal treatment for autoantibody-mediated aHUS. Eculizumab might be inefficacious for aHUS patients having *DGKE* and *INF2* variants[64, 73].

In Japan, eculizumab was approved for treating complement-mediated aHUS in 2013, and its efficacy and safety have been established in both children[68, 93, 94] and adults[95-97]. In the analysis of 118 Japanese patients with aHUS, 72% of patients received plasma therapy and 42% were treated with eculizumab[22]. The prognosis of Japanese patients was relatively favorable compared to Caucasian patients, and no significant differences were found between the outcomes of patients treated with eculizumab and without eculizumab. This is probably due to the unique genetic background of the Japanese population, the predominance of C3 p.I1157T. Of note, it has been revealed that the C5 variant at position Arg885 impairs eculizumab efficacy, and was detected in 3.2% of paroxysmal nocturnal hemoglobinuria patients[98]. No patients have been reported in aHUS until now, but we should consider the screening of this polymorphism when eculizumab is inefficacious for aHUS patients.

Conclusions

Recent progress in the field of aHUS has revealed that the diverse factors were associated with aHUS. It

remains unclear, however, whether the genetic variants unrelated to the complement system lead to uncontrolled complement activation or not. Actually, some patients with variants in *DGKE* or *INF2* showed poor responses to anti-complement therapy, suggesting that specific treatment strategy may be needed according to individual abnormalities. Furthermore, the underlying complement abnormalities have also been detected in other types of TMAs, such as HSCT-TMA and pregnancy-related complication. However, it is also likely that this kind of coincidence should be extremely rare, and further studies are needed to elucidate the efficacy of complement inhibition for these subgroups with the confirmation underlying complement abnormalities by genetic and biological test.

Epidemiological and genetic studies of Japanese patients with aHUS have revealed that the distinct genetic background of Japan, high frequencies of C3 variants and low risk of carrying CFH variants. Moreover, a prevalent C3 p.I1157T is associated with favorable prognosis in spite of high recurrence rate. These data would be helpful to determine treatment strategies for Japanese patients with aHUS. Our institution, division of Nephrology and Endocrinology, the University of Tokyo Hospital, has been performing the cohort analysis of patients with aHUS. Consultation for diagnostic test of aHUS is available by e-mail (ahus-office@umin.ac.jp).

Conflict of Interest

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