and thrombomodulin (THBD)) [4–7]. Additionally, gain-of-function mutations of key complement component C3 and complement factor B (CFB) [8, 9] have been found to predispose to aHUS.

However, the pathophysiology of TTP, which has been largely elucidated in recent years, involves an imbalance between the levels of von Willebrand factor and its cleaving protease, a disintegrin-like and metalloprotease with thrombospondin-1 repeats 13 (ADAMTS-13). This imbalance leads to the presence of large multimers of von Willebrand factor, which then bind platelets and form thrombi in various organs. Low activity of the cleaving protease has been noted in adults with antibodies to ADAMTS-13 [10]. Congenital defects in the ADAMTS-13 gene lead to low levels of the protease; this is the most common cause of TTP in children.

Multiple conditions have been associated with aHUS, including infections, certain drugs, autoimmune conditions, transplantation, pregnancy, and metabolic conditions [11, 12]. However, aHUS occurring in the nontransplant postsurgical period has rarely been reported.

2. Case Presentation

An 8-month-old boy was referred to the Department of Pediatric Cardiovascular Surgery in our center for surgical repair of tetralogy of Fallot. His initial preoperative blood count and complete chemistry results were normal. His complement levels were not examined. The patient was transferred to the pediatric intensive care unit for observation and further management. On postoperative day 1, he was found to have anemia with a hemoglobin level of 10.6 g/dL and thrombocytopenia with a platelet count of 21,000/mm^3 (Table 1(a)). The patient's prothrombin time-international normalized ratio (PT-INR) and activated partial thromboplastin time (APTT) were prolonged at 1.47 and 30.10 seconds, respectively. The patient was diagnosed with disseminated intravascular coagulation (DIC) and treated with fresh frozen plasma (FFP) and a platelet transfusion. Macrohematuria developed on postoperative day 8, and FFP and platelet transfusions were performed repeatedly to control his bleeding. However, his renal function and consciousness level only temporarily improved; disturbance of consciousness and renal dysfunction redeveloped on postoperative days 16 to 24 (Figure 1).

On postoperative day 25, physical examination demonstrated a blood pressure of 90/57 mmHg and a pulse of 105/min. Laboratory examination showed only mild anemia with a hemoglobin level of 11.5 g/dL as well as thrombocytopenia with a platelet count of 13,000/mm^3. Schizocytes were observed in the patient's blood smear, and a low haptoglobin level (<10 mg/dL) was noted. The patient's PT-INR and APTT were prolonged at 2.33 and 45.0 seconds, respectively. Blood chemistry results disclosed renal failure, with a creatinine and blood urea nitrogen level of 1.18 and 108.00 mg/dL, respectively. His complement levels (reference ranges) were as follows: C3, 40 mg/dL (69–128 mg/dL); C4, 12.7 mg/dL (14–36 mg/dL); and CH50, 19.5 U/mL (25–50 U/mL). His other blood chemistry data were as follows: total protein, 4.9 g/dL; albumin, 2.8 g/dL; total bilirubin, 2.07 mg/dL; serum aspartate aminotransferase, 126 IU/L; and serum alanine aminotransferase, 8 IU/L (Tables 1(a) and 1(b)). Urinalysis showed proteinuria and hematuria. The patient had neither diarrhea nor bloody stool. Stool culture results were negative for *E. coli* O157. According to the above data and the patient's neurological disturbances, acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia, TMA (most likely aHUS) was considered as the working diagnosis. On postoperative day 26, brain CT was performed to identify the cause of the persistent disturbance of consciousness and showed severe, extensive brain edema. Furthermore, pupillary light reflex deficits were observed. The patient was not expected to recover without neurological sequelae. Therefore, continuous hemodiafiltration, peritoneal dialysis, and plasma transfusion were performed as conservative therapy. The patient died on postoperative day 50.

Table 1: (a) Time series of patient's laboratory data. (b) Specific data of the patient for aHUS (POD25).

(a)

Parameters (unit of measurement)	−1POD	1POD	25POD	Normal range
White blood cell (/mm^3)	14700	3700	29900	4000–9000
Hemoglobin (g/dL)	14.3	10.6	11.5	11.0–15.0
Platelet (×10^4/mm^3)	35.4	2.1	1.3	16.1–36.0
Albumin (g/dL)	4.8	3.0	2.8	3.9–4.9
AST (U/L)	73	294	126	12–29
ALT (U/L)	38	25	8	5–29
LDH (U/L)	292	1167	2147	106–220
Total bilirubin (mg/dL)	0.62	2.31	2.07	0.4–1.3
BUN (mg/dL)	9	9	108	9–21
Creatinine (mg/dL)	0.18	0.33	1.18	0.80–1.30
Haptoglobin (mg/dL)			<10	19–170
Schizocyte			+	(−)
PT-INR	1.03		2.33	1.0
APTT (s)	19.9		46.0	20–30
FDP (μg/mL)	2		42	0–5

(b)

Parameters (unit of measurement)	Results	Normal range
C3 (mg/dL)	40.0	86–160
C4 (mg/dL)	12.7	17–45
CH50 (IU/mL)	19.5	35–45
CFH gene mutation	Not detected	Not detected
CFI gene mutation	Not detected	Not detected
CFB gene mutation	Not detected	Not detected
MCP gene mutation	Not detected	Not detected
C3 gene mutation	R425C	Not detected
THBD gene mutation	Not detected	Not detected
Anti-FH antibody	Not detected	Not detected
ADAMTS-13 activity assay	Normal	Normal

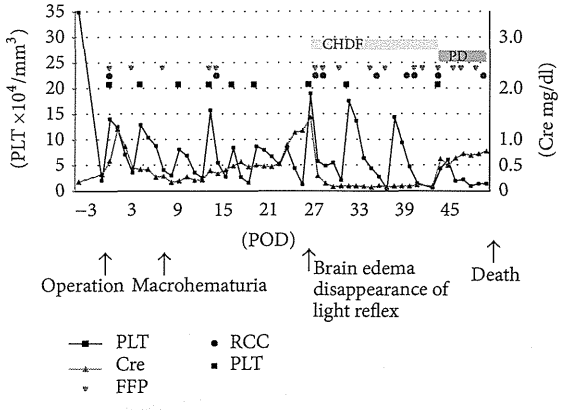

FIGURE 1: Laboratory findings during the clinical course.

Further evaluation revealed that his CFH level was normal, and a hemolytic assay using the patient's serum and that of normal controls showed no significant difference. Anti-FH antibodies were not detected in the patient's plasma. Sequencing of the CFH, CFI, MCP, CFB, and THBD genes was normal. His ADAMTS-13 activity was also normal (Table 1(b)). However, he had a potentially causative mutation (R425C) in the b-chain of C3 in exon 12. This finding confirmed that the patient had aHUS caused by a C3 gene mutation.

The patient's parents gave consent for C3 gene analysis in the patient, his elder sister, and his aunt (Figure 2(a)). Restriction fragment length polymorphism analysis confirmed that his father and aunt had this same mutation (Figure 2(b)). The patient's father and aunt had no history of any surgical procedures, although they developed the common cold at a typical frequency. Furthermore she had not become pregnant before then.

3. Discussion

We have presented a case of aHUS that developed in an infant after cardiac surgery for repair of tetralogy of Fallot. aHUS has been used to classify any HUS not caused by Shiga toxin. A variety of precipitating events have been associated with aHUS, including infections, drugs, autoimmune conditions, vaccination, malignancy, organ transplantation, pregnancy, and metabolic conditions [11, 12]. Although it is an uncommon postoperative complication, aHUS must be considered as a possible cause of acute kidney injury after surgical procedures [13]. Above all, the alternative complement pathway plays a key role in the pathogenesis of aHUS [11, 12]. Mutations in CFH account for approximately 25% of the genetic predisposition to aHUS [11, 14]. Mutations in CFI and MCP account for 5% to 10% and 10% of cases of aHUS, respectively [11, 15]. Mutations in C3 have been reported in several cohorts of patients with aHUS at a frequency of 4% to 10% [12, 16, 17].

Mutations of complement component C3 have been described more recently. C3 is cleaved to form the anaphylatoxins C3a and C3b, which are highly reactive and can bind to cell surfaces via their reactive thioester. C3b then can interact with CFB in the presence of factor D to form the alternative pathway of complement C3 convertase (C3bBb), which further cleaves C3, introducing a positive-feedback loop. Initial functional analysis showed that MCP was unable to bind to mutant C3, preventing its cleavage to iC3b [8]. Two C3 mutations that result in decreased secretion have been described, but their pathogenetic role remains uncertain. More recently, two mutations in C3 that bind to CFB with higher affinity and cause increased C3 convertase formation have been reported [18, 19]. These mutations result in increased complement activation on platelets [18] and the glomerular endothelium [19].

The underlying pathogenesis of TMA is considered to involve endothelial cell injury that results in renal arteriolar peritubular capillaries, intracapillary platelets, and fibrin-rich thrombi formation [20]. It is speculated that the pathogenesis of postoperative TTP involves massive endothelial damage during surgery [21]. In the present case, the endothelial cells might have been damaged during cardiac surgery. The presence of severe thrombocytopenia (platelet count $\leq 2.1 \times 10^4/\mu L$), anemia, and hemolytic parameters (elevated LDH and bilirubin levels) was observed immediately after surgery. These findings might be clues to the presence of aHUS that developed hyperacutely (Figure 1).

Fan et al. [22] recently reported the cause of aHUS in 10 Japanese patients. Eight cases were sporadic and the other two arose from one family. They identified 7 causative or potentially causative mutations in CFH (p.R1215Q), C3 (p.R425C, p.S562L, and p.I1157T), membrane cofactor protein (p.Y189D and p.A359V), and THBD (p.T500M) in 8 of the 10 patients. The patient with the n p.R425C mutation was our patient in the present report. Two mutations, p.R425C and p.S562L, are novel, and the p.I1157T mutation has been previously reported in the United States and Spain [16].

Fan et al. [22] described another patient with C3 mutation for whom surgery became the probable triggering event. The patient also had C3 p.I1157T, developed aHUS after undergoing nephrectomy at 70 years of age, and was treated with hemodialysis [23]. They stated that, in addition to the main genetic mutation, environmental factors and/or other genetic variations were likely required for the manifestation of aHUS as a secondary hit [23]. The cardiac surgery and/or presence of DIC might be secondary hits because other triggering events such as respiratory infection, diarrhea, aHUS-inducing drugs, and others were not demonstrated in our patient. Additionally, although our patient's father and aunt were affected by aHUS, they had heterozygous C3 mutation pR425C. This may be why they have not yet encountered a secondary hit.

As mentioned above, a variety of precipitating events are thought to contribute to the development of aHUS [11]. It is often reported that kidney transplantation is also a causative factor in aHUS [24]. However, there has been only one report describing the onset of aHUS initiated by a surgical procedure other than transplantation [13]. In that report, a 66-year-old woman developed renal impairment on the first day after laparoscopic hemicolectomy. aHUS is

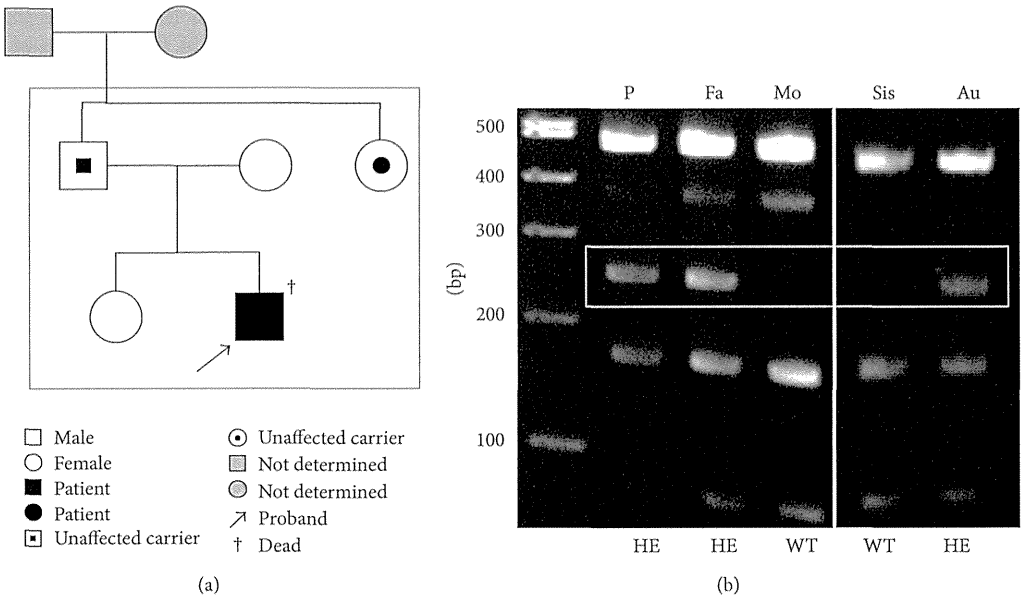

FIGURE 2: (a) The pedigree of the patient. (b) RFLP analysis. P: proband, Fa: father, Mo: mother, Sis: sister, Au: aunt, WT: wild type, and HE: heterozygote.

an uncommon postoperative complication; however, considering its different treatment modalities and poor outcomes, aHUS must be considered as a possible cause of acute kidney injury combined with thrombocytopenia and anemia after surgical procedures [13]. Conversely, TTP occurring in the nontransplantation postsurgical setting has been reported several times and has often been described after both cardiothoracic and vascular surgeries and noncardiovascular surgeries [25–28]. To the best of our knowledge, this is the first case report of postoperative aHUS due to a complement C3 mutation after nontransplantation surgery. In postsurgical patients who develop unexplained microangiopathic hemolytic anemia and thrombocytopenia, the diagnosis of aHUS should be considered and plasma exchange should be contemplated while other causes such as infection and disseminated intravascular coagulation are evaluated.

Conflict of Interests

Dr. Yoshihiro Fujimura received a grant for collaborative research from Alexion and a research grant from Takeda Medical Foundation. The other authors declare no conflict of interests regarding the publication of this paper.

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ORIGINAL ARTICLE

Analysis of patients with atypical hemolytic uremic syndrome treated at the Mie University Hospital: concentration of *C3* p.I1157T mutation

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Abstract Atypical hemolytic uremic syndrome (aHUS) is caused by abnormalities of the complement system and has a significantly poor prognosis. The clinical phenotypes of 12 patients in nine families with aHUS with familial or recurrent onset and ADAMTS13 activity of ≥20 % treated at the Mie University Hospital were examined. In seven of the patients, the first episode of aHUS occurred during childhood and ten patients experienced a relapse. All patients had renal dysfunction and three had been treated with hemodialysis. Seven patients experienced probable triggering events including common cold, influenza, bacterial infection and/or vaccination for influenza. All patients had entered remission, and renal function was improved in 11 patients. DNA sequencing of six candidate genes, identified a *C3* p.I1157T missense mutation in all eight patients in six families examined and this mutation was causative for aHUS. A causative mutation *THBD* p.D486Y was also identified in an aHUS patient. Four missense mutations, *CFH* p.V837I, p.Y1058H, p.V1060L and *THBD* p.R403K may predispose to aHUS manifestation; the remaining seven missense mutations were likely neutral. In conclusion, the clinical phenotypes of aHUS are various, and there are often trigger factors. The *C3* p.I1157T mutation was identified as the causative mutation for aHUS in all patients examined, and may be geographically concentrated in or around the Mie prefecture in central Japan.

Keywords aHUS · C3 mutation · Trigger factor · Renal failure · Thrombotic microangiopathy

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Introduction

Hemolytic uremic syndrome (HUS) [1] is characterized by the presence of microangiopathic hemolytic anemia, thrombocytopenia, renal impairment with symptoms similar to those of thrombotic thrombocytopenic purpura (TTP) [2–4]. Approximately 10 % of cases are classified as atypical due to the absence of Shiga toxin-producing bacterial infection as a trigger [5]. Compared to typical HUS, atypical HUS (aHUS) is considered to be caused by abnormalities of the complement system and has a much poorer prognosis and higher mortality, with up to half of patients progressing to end-stage renal disease [6].

The alternative pathway of the complement system is a natural defense system against invasive microbial attack, in which complement component C3 (C3), the central complement protein, is hydrolyzed to C3b and directly binds to the microbe for opsonization or the subsequent activation of the complement pathway [7]. When C3b binds to host cells, further activation of the complement system is stringently limited by several endogenous complement regulatory proteins present on the surface of the host cells [8]. Complement factor H (CFH) and membrane cofactor protein (MCP or CD46) are cofactors for the proteolytic degradation of C3b by complement factor I (CFI). Thrombomodulin, an endothelial anticoagulant glycoprotein encoded by *THBD*, also functions as a cofactor for CFI-mediated C3b inactivation [9]. The uncontrolled activation of the alternative pathway of the complement system plays an important role in the pathogenesis of aHUS. More than half of patients with aHUS have mutations in the genes involved in the alternative pathway of the complement system [5]. Mutations with loss-of-function of regulators (CFH, CFI, MCP and THBD) [9–12] and gain-of-function of key complement components (C3 and CFB) [13, 14] have been found to predispose patients to the development of aHUS. A normal plasma level of complement proteins does not preclude the presence of mutations in these genes. More importantly, genotype–phenotype correlations of aHUS have clinical significance in predicting renal recovery and transplant outcomes [12].

We previously reported the clinical characteristics and genetic variations of ten aHUS patients in nine family, in whom two aHUS patients in one family have been treated at the Mie University Hospital [15]. In the present study, we examined the clinical phenotypes of 12 aHUS patients in nine families, including previously reported those two patients in one family, all treated at the Mie University Hospital. We also performed the genetic analysis in six aHUS patients, in total, identified at the Mie University Hospital. Unexpectedly, we found that all six patients shared the same genetic mutation, *C3* p.I1157T missense mutation.

Materials and methods

Twelve aHUS patients in nine families, all treated at the Mie University Hospital, were investigated in this studies. We included two previously reported patients (patients JJ1 and JJ2 [15]) in one family as patients IV-1 and IV-2, respectively, because they have been treated at the Mie University Hospital. Six of the patients were sporadic and three were familial. The diagnosis of aHUS was made based on the simultaneous occurrence of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure without Shiga toxin [16]. In addition, patients with familial or recurrent aHUS, which is associated with an ADAMTS13 activity of more than 20 % to completely exclude TTP due to ADAMTS13 deficiency, and a survival of more than 1 year, were selected in this study. The study protocol was approved by the Mie University Graduate School of Medicine and the National Cerebral and Cardiovascular Center, and written informed consent was obtained from all of the participants.

ADAMTS13 activity assay

The ADAMTS13 activity was measured using a FRETS-VWF73 peptide (Peptide Institute, Japan) according to the method reported by Kokame et al. [17, 18].

Hemolytic assay

The hemolytic assay was performed at the Department of Blood Transfusion Medicine, Nara Medical University [15]. Resuspended sheep red blood cells (Japan Lamb, Japan) were incubated with a dilution series of a patient plasma sample at 37 °C for 30 min, and the level of hemoglobin released from the red blood cells was measured by the absorbance at 414 nm [19]. The hemolysis obtained from normal plasma spiked with monoclonal antibody against CFH (200 μg IgG/ml, final) was defined as a 100 % hemolysis as the control. The result of hemolysis in patient plasma was expressed as follows; enhanced ($\geq$50 % of the control), moderate (15–50 %) and no hemolysis (<15 %).

Mutation screening

Genomic DNA was extracted from the peripheral blood leukocytes. The coding exons and the intronic flanking regions of *CFH* (NM 000186.3), *C3* (NM 000064.2), *MCP* (NM 002389.4), *CFI* (NM 000204.3), *CFB* (NM 001710.5) and *THBD* (NM 000361.2) were sequenced at Department of Molecular Pathogenesis, National Cerebral and Cardiovascular Center, as previously described [15]. The A of the ATG translation initiation start site was designated as

+1 position and the initial Met was denoted as +1. Multiplex ligation –dependent probe amplification analysis was used to screen the gene deletion using a commercially available kit (MLPA kit P236-A2, MRC-Holland, the Netherlands) as previously described [15].

Results

The clinical features of 12 aHUS patients in nine families are summarized in Table 1. All of the patients showed no signs for infection with Shiga toxin-producing *Escherichia coli*. Nine families were non-consanguineous with each other. The first episode of aHUS occurred during childhood (≤10 years of age) in seven patients, while five patients experienced their first episode at more than 20 years of age. Ten patients had experienced relapse, with a varying number of relapse events. All patients had renal dysfunction and three patients had been treated with hemodialysis (HD), although they were being weaned from this treatment. Three patients had central nervous symptoms. Seven patients experienced probable triggering events, such as the common cold, influenza, bacterial infection or vaccination for influenza.

The laboratory data of 12 patients with aHUS are summarized in Table 2. The platelet count was markedly reduced in all patients, with the exception of patient IV-2 and hemoglobin levels, ranging from 5.9 to 9.8 g/dl. The levels of creatinine and lactate dehydrogenase were increased in most patients with aHUS, while the total bilirubin levels were slightly increased. The plasma ADAMTS13 activity was within the range of 40–100 % in all patients. The patients were treated with plasma exchange, transfusion of fresh frozen plasma, steroid or infusion therapy or the administration of anti-platelet, anti-hypertensive or antibiotic agents. All patients had remission, in addition, the renal function improved in 11 patients and worsened in one patient (IV-2). "When patient VI-1 developed relapse, he was treated with eculizumab and his symptoms promptly improved.

Genetic analyses of six candidate genes and the gene deletion have been performed in six aHUS patients and found that all patients had a causative mutation, p.I1157T, in *C3*, and the same mutation has been previously identified in two aHUS patients in one family (patients IV-1 and IV-2) treated at the Mie University Hospital, as summarized in Table 3. A causative mutation *THBD* p.D486Y previously identified in aHUS patients in Europe and North America [7] was also identified in an aHUS patient, I-2. Gene deletion of *CFH* and *CFHRs* were not found in the aHUS patients. DNA sequencing identified additional 12 missense mutations. Among them, two rare missense

Table 1 Subjects

	Age	Sex	Age of first episode	Relapse	Outcome	Renal dysfunction	HD	CNS symptoms	Trigger for aHUS
I-1	38	F	6	6	Survive	Positive	ND	Positive	Common cold
I-2	68	F	20	2	Survive	Positive	ND	Negative	–
II	35	F	5	6	Survive	Positive	ND	Negative	Influenza
III	12	M	1	2	Survive	Positive	Weaning	Negative	Common cold, infection
IV-1	36	M	2	7	Survive	Positive	Weaning	Positive	Common cold, infection
IV-2	71	M	70	0	Survive	Positive	Weaning	Negative	–
V	38	F	21	3	Survive	Positive	ND	Positive	Common cold, vaccine[a]
VI-1	9	M	9	1	Survive	Positive	ND	Negative	Infection
VI-2	45	M	38	0	Survive	Positive	ND	Negative	–
VII	2	M	1	1	Survive	Positive	ND	Negative	–
VIII	22	F	3	7	Survive	Positive	ND	Negative	–
IX	28	M	24	7	Survive	Positive	ND	Negative	Common cold

IV-2 the father of IV-1, *HD* hemodialysis, *ND* not done, *CNS* central nerve system

[a] Vaccine for influenza

Table 2 Laboratory data of the patients with aHUS

	Platelet ($\times 10^8$/ml)	Hemoglobin (g/dl)	Creatinine (mg/dl)	LDH (U/l)	T-Bil (mg/dl)	ADAMTS13 (%)
I-1	1.9	7.4	1.7	972	1.9	76.3
I-2[a]						100
II	3.5	5.9	4.7	4485	3.5	88.8
III	2.6	5.9	4.7	4465	2.4	53.8
IV-1	2.8	6.7	10.6	1280	1.1	92.5
IV-2	9.5	9.6	8.0	398	1.2	96.0
V	3.5	9.8	1.4	928	3.5	92.5
VI-1	1.4	6.6	1.8	3160	1.4	67.5
VI-2	1.0	7.4	1.9	2850	1.8	40.0
VII	2.6	7.2	10.9	696	2.6	97.5
VIII	2.2	6.0	0.85	1098	2.2	100
IX	1.5	7.9	2.1	1780	1.9	ND

T-Bil total bilirubin, *ND* not done

[a] Previous data not available

mutations, *CFH* p.Y1058H and p.V1060L, and two low-frequency missense mutations, *CFH* p.V837I and *THBD* p.R403K, might predispose to aHUS, and the remaining seven missense mutations were likely neutral. Patient IV-1 who has *C3* p.I1157T developed aHUS at 2 year of age and experienced seven recurrences of aHUS (Table 1). He had acute renal failure and was treated with HD. Patient IV-2, a patient IV-1's father, who also has *C3* p.I1157T, developed aHUS after undergoing nephrectomy at 70 years of age and was treated with HD. Both patients and patient VI exhibited a mildly elevated hemolytic activity, however other five patients with *C3* p.I1157T did not show an elevated activity (Table 3).

Discussion

In Japan, the frequency of Shiga toxin-producing *Escherichia coli* (STEC)-HUS is approximately 40 % of all cases of thrombotic microangiopathy (TMA), according to the national questionnaire survey of TMA [20, 21], indicating that the frequency of STEC-HUS is lower in Japan than in Europe and North America. There are few methods for detecting abnormalities in the regulatory complement system. Therefore, the hematologists diagnosed the patients with suspected aHUS as having TTP. Other doctors also diagnosed the patients as having renal disease due to TMA. Therefore, there are a few reports of aHUS in Japan. In the present study, cases of fatal TMA were excluded due to the difficulty of confirming the subject's past and family history. Although all patients evaluated in the present study survived, the outcomes of aHUS were not always good. It has recently been reported that the terminal complement inhibitor eculizumab, which is approved for the treatment of paroxysmal nocturnal hemoglobin-uria, improves aHUS [22]. Eculizumab was also approved for the treatment of aHUS in Japan in September 2013. The drug binds with high affinity to human complement protein C5 and blocks the generation of proinflammatory C5a and the membrane attack complex, C5b-9 [22, 23]. "Although eculizumab is expensive, its use promptly improved the relapse in patients VI-1. Patients III, IV-1 and IV-2, who had been tread with HD, should receive eculizumab if they exhibit a relapse. Treatment with eculizumab is recommended in the acute phase in patients with mild aHUS, such as that involving C3 mutations, and as prophylaxis in those with severe aHUS, such as that involving CFH mutations [24].

In the present study, we examined the genetic abnormalities in six aHUS patients and identified the *C3* p.I1157T mutation as a causative mutation in the patients. We have previously performed the genetic analysis in aHUS patients and identified the same mutation in three patients, two of whom were referred from the Mie University Hospital [15]. Thus, as summarized in Table 3, eight aHUS patients treated at the Mie University Hospital carried the *C3* p.I1157T mutation. This finding was unexpected because our previous genetic analysis in 10 aHUS patients revealed the heterogeneous phenotype-genotype correlation [15]. The *C3* p.I1157T mutation might be geographically concentrated in or around the Mie prefecture located in central Japan.

A causative mutation *THBD* p.D486Y previously identified in aHUS patients in Europe and North America was also identified in the present study. The allele frequency of this mutation was 0.011 in the Japanese population referred from the 1000 Genomes database [25] and the recombinant mutant thrombomodulin with this mutation showed defective C3b inactivation [9]. The significance of remaining missense mutations was unknown. Both p.Y1058H and p.V1060L in the short consensus repeat-18 domain of CFH were rare mutations. They were not

Table 3 Genetic analysis of eight aHUS patients in the six families

Patients			I-1	I-2	II	III	IV-1	IV-2	V	VI
Hemolytic assay			−	−	−	−	±	±	−	±
Missense mutations	rs number	MAF								
CFH										
c.184G>A	rs800292	0.416				p.V62I			p.V62I	p.V62I
c.2509 G>A	rs55807605	0.011						**p.V837I**		
c.2808G>T	rs1065489	0.455	p.E936D (homo)	p.E936D	p.E936D (homo)	p.E936D	p.E936D (homo)	p.E936D	p.E936D	p.E936D
c.3172T>C	rs55679475	None			**p.Y1058H**	**p.Y1058H**				**p.Y1058H**
c.3178G>C	rs55771831	None			**p.V1060L**	**p.V1060L**				**p.V1060L**
CFI										
c.603A>C	rs145769028	0.028					p.R201S	p.R201S		
c.1217G>A	rs74817407	0.096				p.R406H				
C3										
c.3470T>C		None	**p.I1157T**	**p.I1157T (homo)**	**p.I1157T**	**p.I1157T**	**p.I1157T**	**p.I1157T**	**p.I1157T**	**p.I1157T**
CFB										
c.94C>T	rs12614	0.112	p.R32W	p.R32W	p.R32W		p.R32W	p.R32W (homo)		
c.95G>A	rs641153	0.073								p.R32Q
THBD										
c.1208G>A	rs41400249	0.006							**p.R403K**	
c.1418C>T	rs1042579	0.253				p.A473V			p.A473V	
c.1456G>T	rs41348347	0.011		p.D486Y						
CNV of CFH and CFHRs					Normal	Normal	Normal	Normal	Normal	

Hemolytic assay, − no hemolysis; ± moderate hemolysis. Bold and underlined: definite causative mutation [13], Bold: rare and low-frequency potentially predisposing mutation; homo, homozygote, The A in the ATG translation initiation start site is designated as the +1 position and the initial Met denotes +1

MAF minor allele frequency taken from the 1000 genome database (http://www.1000genomes.org/data), *CFH* complement factor H, *CFI* complement factor I, *C3* complement component 3, *CFB* complement factor B, *THBD* thrombomodulin, *CNV* copy number variation, *CFHRs* CFH related genes

identified in the 1000 Genomes database [25] but were found as somatic mutations in human cancers in the COSMIC database [26]. Both p.V837I in the short consensus repeat-14 domain of CFH and p.R403K in the EGF-like 4 domain of THBD were low-frequency mutations with the minor allele frequency of 0.011 and 0.006, respectively [25]. It can be assumed that, in addition to the main genetic mutation, *C3* p.I1157T, the environmental factors and/or other genetic variations are required for the manifestation of aHUS as a second hit. Rare or low-frequency missense mutations in the aHUS patients identified in the present study may predispose to the aHUS manifestation. The remaining seven missense mutations were likely neutral.

Sheep red blood cells are rich in sialic acid and are capable of binding CFH from the plasma to protect themselves against human complement attack. Therefore, the hemolytic assays are frequently used to evaluate the function of CFH-related abnormalities [18, 27]. Generally, plasma samples containing CFH mutant with mutation in the C-terminal domains or auto-antibodies against CFH would exhibit an increased hemolytic activity. In the present study, patients with the *C3* p.I1157T mutation showed negative and weak hemolytic activity, indicating that this mutation does not directly influence the hemolytic assay.

In conclusion, we found that the clinical phenotypes of aHUS patients are various, and there are often trigger factors. The *C3* p.I1157T mutation was found in eight aHUS patients examined as the causative mutation for aHUS, and would be geographically concentrated in or around the Mie prefecture in central Japan.

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Conflict of interest All authors have no conflict of interest to report.

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ORIGINAL ARTICLE

Eculizumab in the treatment of atypical hemolytic uremic syndrome in an infant leads to cessation of peritoneal dialysis and improvement of severe hypertension

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Abstract
Background Severe hypertension (HTN) and acute kidney injury frequently associated with atypical hemolytic uremic syndrome (aHUS) were refractory to various therapies in the pre-eculizumab era. Here we report the case of a 4-month-old boy who developed aHUS presenting with undetectable C3 protein, no predisposing mutations in complement factors, and no antibodies against factor H.
Methods Repeated plasma infusions and nine sessions of plasmapheresis were ineffective. The patient initially required continuous hemodiafiltration and thereafter peritoneal dialysis. Despite vigorous antihypertensive treatment and improved fluid overload with dialysis, HTN persisted. His low C3 level (<20 mg/dl) suggested unrestricted complement activation. Therefore, based on the suspicion of unrestricted complement cascade in the pathogenesis, treatment with eculizumab, a human anti-C5 monoclonal antibody, was initiated with the aim of controlling disease activity.
Results Eculizumab therapy resulted in the control of severe HTN and cessation of peritoneal dialysis.
Conclusions This infant with HTN and acute kidney injury associated with aHUS was treated successfully with eculizumab.

Keywords Plasmapheresis-resistant · Meningococcal infection · Prophylactic antibiotics · Vaccination · Pathological findings · Atypical hemolytic uremic syndrome · Anti-C5 therapy

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Introduction

Hemolytic uremic syndrome (HUS) is a rare disease with manifestations of microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment. There are two types of HUS: HUS which follows a diarrheal infection often caused by Shiga toxin-producing *Escherichia coli* (Stx-HUS) and atypical HUS (or non-Stx-HUS) caused by one or more genetic mutations. The latter type is extremely rare—accounting for less than 10 % of all HUS cases—and the clinical outcome is unfavorable, with up to one-half of cases progressing to end-stage renal failure and one-fourth of patients dying in the acute phase if they do not receive rigorous treatment [1]. Historically, the first-line therapy for aHUS has been plasma therapy, including plasmapheresis [2, 3]. However, approximately 80 % of patients with aHUS have only partial responses to short-term plasmapheresis with subsequent persistent tissue damage [4]. The main pathogenesis of aHUS is unrestricted overactivation of the alternative pathway of complement activation [5], which has led to eculizumab, a human anti-C5 monoclonal antibody, being used for both

plasmapheresis-resistant and plasmapheresis-dependent cases of aHUS [6–9].

We here report a 4-month-old male baby with plasmapheresis-resistant aHUS who was successfully treated with eculizumab. The results of this very effective therapy was control the patient's extremely severe hypertension and cessation of peritoneal dialysis.

Case report

A severely ill 4-month-old male baby weighing 7.1 kg presented with a 2-day history of fever and vomiting. His father and mother were alive and in good health; his maternal grandmother and grandaunt had died of cerebral infarction, but no details were available. Laboratory tests at a local hospital revealed anemia (8.9 g/dl), thrombocytopenia (64×10^9/l), and acute renal insufficiency consistent with HUS. He was transferred to Hiroshima University Hospital where upon admission, schistocytes on the peripheral blood film, elevated levels of lactate dehydrogenase (LDH; 1,830 U/l), creatinine (1.95 mg/dl), and urea (38.8 mg/dl), and decreased platelets level (41×10^9/l) were detected. Stool culture was negative, and immunoglobulin M (IgM) antibody for O-157 lipopolysaccharide was not detected. A tentative diagnosis of aHUS was made, which was later confirmed by a normal test result for ADAMTS13 (72 %) activity. After admission to the hospital, the patient received multiple blood transfusions, including fresh frozen plasma and continuous hemodiafiltration. However, his general condition remained poor, and hemolytic anemia, elevated LDH, and renal failure did not improve.

On day 26 he was transferred to the Hiroshima Prefectural Hospital for closer management of the renal failure. On admission, the serum complement of C3 and C4 was <20 and 14 (normal range 13–35) mg/dl, respectively. On the third day of admission, plasmapheresis with 60 ml/kg of fresh frozen plasma was initiated and performed for 3 consecutive days, in combination with hemodiafiltration, followed by plasmapheresis every other day for the next 2 weeks; however, there was no remarkable improvement of the hemolysis and renal failure. After nine sessions of plasmapheresis, the patient was not in remission from his disease according to the guideline of the European Pediatric Study Group for HUS [2]. Moreover, his severe hypertension (systolic blood pressure 140–150 mmHg), which initially did not respond to fluid removal by hemodiafiltration, was also refractory to the treatment with an intravenous large dose of nicardipine chloride, peroral enarapril, and losartan. At that time, eculizumab was not licensed for the treatment of aHUS in Japan. Therefore, after receiving approval from the institutional ethics committee, we started the patient on eculizumab, with an initial dose of 300 mg administered on day 48, followed by a second dose of 300 mg on day 55; thereafter and up to the present (17 months), the patient has been receiving one 300-mg dose of eculizumab every 3 weeks in accordance with the manufacturer's recommendations, with sustained remission. For the prevention of infection after the treatment with eculizumab, the patient has received continuous antibiotic prophylaxis with cefdinir from the first administration of eculizumab, and on days 62 and 169 he received tetravalent conjugate meningococcal vaccine (Menactra®; Sanofi Pasteur, Lyon, France).

The severe hypertension gradually improved, and the patient was switched from intravenous nicardipine chloride to peroral nifedipine 11 weeks after the first administration of eculizumab. Plasma renin activity on days 40, 60, 84, and 123 was 18, 77, 5.7, and 1.2 ng/ml/h, respectively. Renal function improved, and peritoneal dialysis (PD) was stopped on day 117. On day 108 the patient presented with white stool and jaundice. Liver function tests were consistent with cholestatic jaundice with a total bilirubin of 2.5 mg/dl, direct bilirubin of 1.7 mg/dl, aspartate transaminase (AST) of 1,337 U/l, alanine transaminase (ALT) of 1,667 U/l, LDH of 954 U/l, and gamma-glutamyl transferase (GTP) of 1,093 U/l. Abdominal ultrasonography revealed dilatation of the common bile duct and some gallstones. Magnetic resonance cholangiopancreatograpy confirmed the diagnosis of cholelithiasis, which regressed spontaneously (Fig. 1).

Genetic work-up showed no predisposing mutations in complement factor (CF) H, CFI, CFB, C3, thrombomodulin, or membrane cofactor protein. At the initiation of the first session of plasmapheresis, protein level and CFH activity were normal, and antibodies to CFH were undetectable. Membrane cofactor protein expression on peripheral blood mononuclear cells was within the normal limit. Cobalamin-C disorder was ruled out based on the normal level of homocystine in the urine and negative tandem mass screening test.

A kidney ultrasound on admission to our hospital showed normal-sized kidneys for age with increased echogenicity in the renal cortex. A kidney biopsy performed on day 159 showed mild swelling of the glomerular endothelium and decrease in the size of the lumen, with approximately 10% of interstitial cell infiltration and fibrosis. A small number of fragmented erythrocytes were observed in a few glomeruli among the 47 glomeruli assessed (Fig. 2b). Only rarely were wrinkling, collapse, and/or thickening of the glomerular basement membranes, identical to that observed in ischemic lesions, observed in the biopsy specimen. However, a few glomeruli showed partial duplication of the peripheral basement membrane, which is a marker of chronic change. Moreover, several intralobular arteries and arterioles had a narrowed lumen due to myointimal hyperplasia (Fig. 2a). Juxtaglomerular apparatus hyperplasia was also observed in some glomeruli (Fig. 2a). Immunofluorescence findings showed weak C3, fibrinogen, and IgM depositions in glomerular capillaries.

Currently, 17 months after the initiation of eculizumab administration, the laboratory test results of our patient are

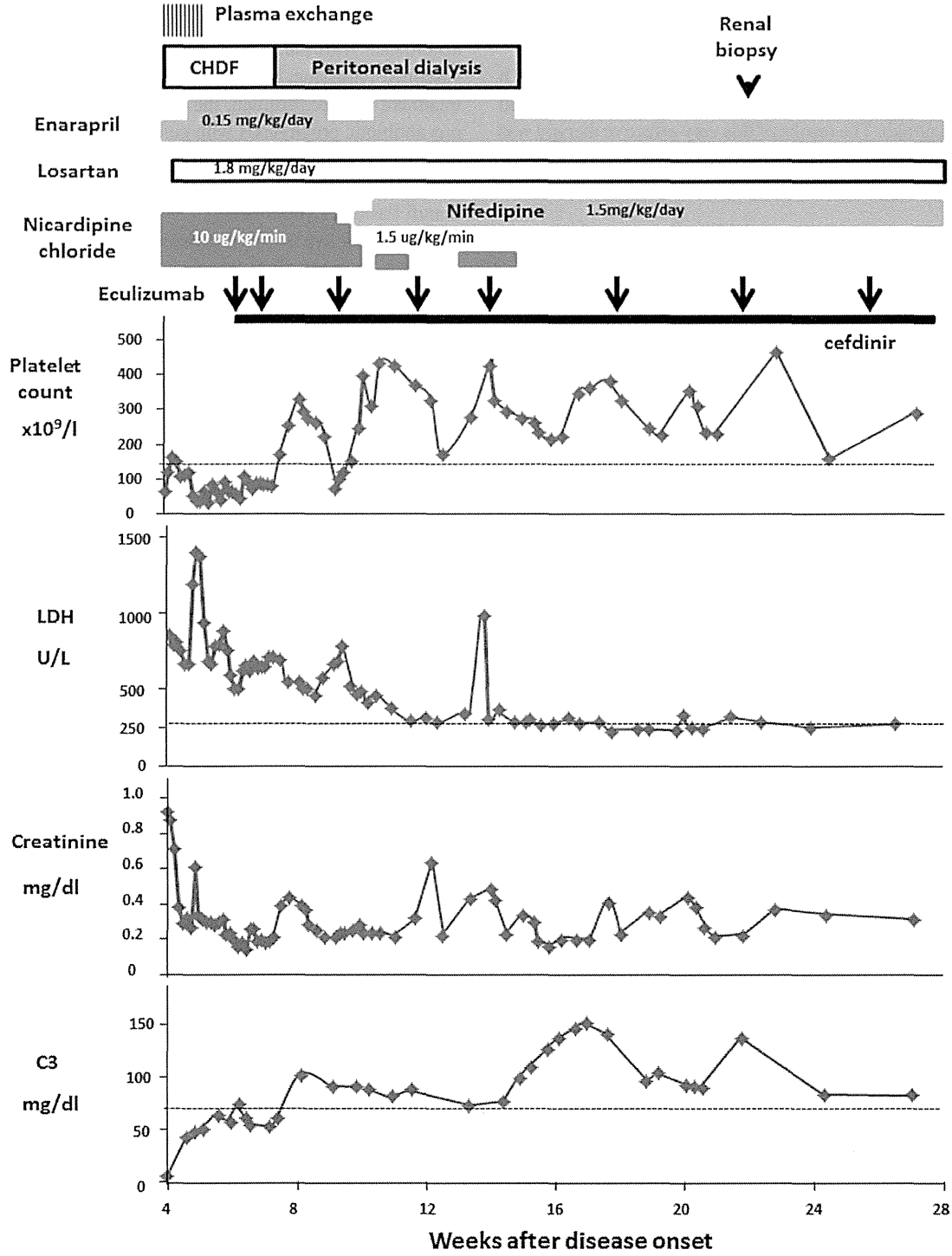

Fig. 1 Clinical course of the patient before and after eculizumab therapy. The patient received 9 sessions of plasmapheresis (*vertical bars*) in combination with continuous hemodiafiltration (CHDF) without clinical remission; severe hypertension continued despite vigorous antihypertensive treatment. including a high dose of nicardipine chloride. After 2 administrations of eculizumab (*vertical arrows*), the platelet count and complement 3 (C3) level increased and the lactate dehydrogenase (LDH) level decreased. The therapy allowed cessation of peritoneal dialysis and nicardipine chloride administration. Renal biopsy (*arrowhead*) was performed on day 159 after disease onset. *Dashed horizontal lines* show the remission level for platelet count (150 × 10^9/l), the upper limit of the reference range for LDH (260 U/l) in our institution, and the lower limit of the reference range for C3 (70 mg/dl)

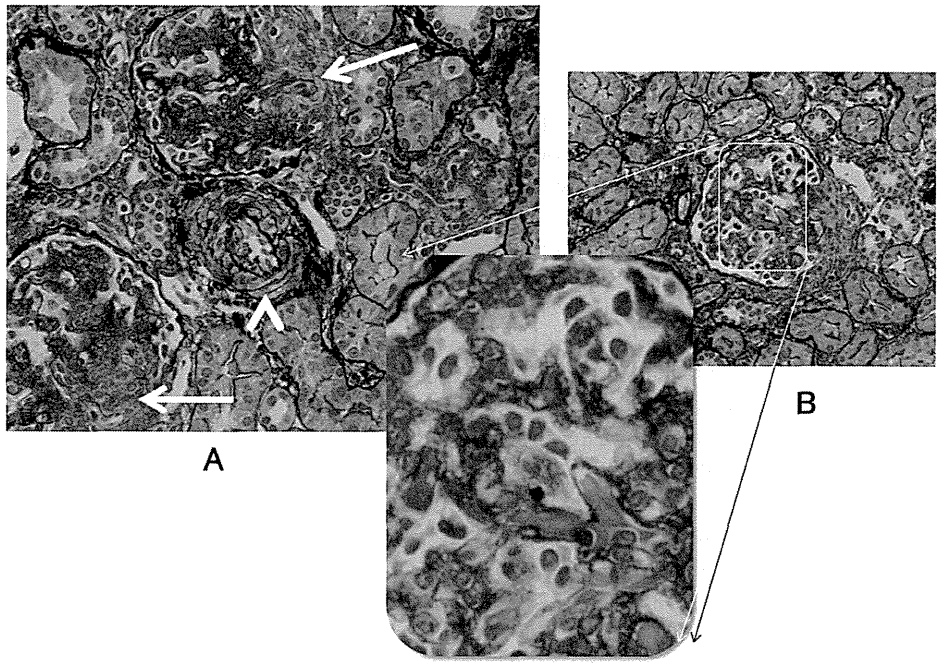

Fig. 2 Pathological findings of renal specimens after hematological remission and cessation of peritoneal dialysis. **a** Periodic acid–methenamine silver (PAM) staining revealed an arteriole with a narrowed lumen due to myointimal hyperplasia (*arrowhead*) and juxtaglomerular apparatus hyperplasia (*arrows*). **b** PAM staining reveals thrombotic microangiopathy with intracapillary erythrocytes

as follows: hemoglobin, 11.4 g/dl; platelet count, 247 × 10^9/l; AST, 34 IU/l; ALT, 20 IU/l; LDH, 289 IU/l; gamma-GTP, 41 IU/l; total bilirubin, 0.1 mg/dl; urea nitrogen, 21.2 mg/dl; creatinine, 0.33 mg/dl {[classified into chronic kidney disease (CKD) stage II according to diagnostic criteria of Japanese children [10]}; Na, 139 mEq/l; K, 4.4 mEq/l; Cl, 104 mEq/l; haptoglobin, 86 mg/dl; C3, 104 mg/dl; urinary protein negative; urine beta2 microglobulin, 172 ug/l.

Discussion

Eculizumab is emerging as an effective treatment for post-transplant aHUS recurrence [11] and may have a certain role in treating de novo aHUS [6–9, 12, 13], halting the hemolytic process. The complement pathway dysregulation that includes loss-of-function mutations in genes of the complement regulatory system and gain-of-function mutations in genes encoding the complement activators factor B and C3 seem to play a crucial role in the pathogenesis of aHUS. The low level of C3 in our patient indicated consumption due to unrestricted complement overactivation. This led us to initiate eculizumab treatment, and the disease activity of the patient subsequently subsided.

Three issues attracted our attention in light of these results. The first of these is the reason why the renal function of our patient improved after eculizumab therapy. Complement-induced endothelial injury might result in stenosis due to endothelial cell swelling and detachment, followed by inflammatory leucocyte recruitment, subendothelial expansion, and reactive smooth-muscle cell proliferation. Moreover, thrombus formation on the injured endothelium also contributes to the stenotic process, leading to renal hypoperfusion and clinical renal failure. The late initiation of eculizumab therapy—not as a first-line therapy—has been shown in previous studies to result in a partial or complete recovery of renal function in patients with aHUS. To date, however, limited data on pathological findings following the improvement of renal function by treatment with eculizumab are available. Pathological data from a few large-scale studies have not provided further insights into the mechanism and process of recovery of renal function [14, 15]. The degree of vascular involvement is well correlated with the ultimate prognosis of thrombotic microangiopathy [16]. In our study, renal biopsy on day 159 showed very mild glomerular intracapillary thrombosis with 10 % of interstitial changes. Moreover, there were few ischemic changes, such as collapse of the capillary tuft with wrinkling and thickening of the capillary basement membranes, which is unlikely due to involvement of relatively large-sized vessels. The narrow extent of arterial and arteriolar damage in this case seems to allow a nearly complete improvement of renal function.

The second issue is the timing that the therapy should be initiated. The switch from plasma therapy to eculizumab has been shown to improve renal function even in patients with long-lasting and stable CKD. Kim et al. [7] reported a 7-month-old girl with aHUS dependent on plasmapheresis and PD who was able to stop PD after eculizumab therapy. This patient received eculizumab 15 weeks after the initiation of PD. In prospective phase 2 trials in which aHUS patients aged ≥12 years received eculizumab for 26 weeks, a number of dialysis-dependent patients were ultimately able to

stop dialysis [14]. In these trials, eculizumab inhibited complement-mediated thrombotic microangiopathy and was associated with significant time-dependent improvement in renal function in patients with aHUS [14, 15]. Despite the lack of evidence from randomized controlled studies, data from published studies indicate that eculizumab is likely to achieve better control of aHUS than dose plasma therapy. Based on these data, Zuber J et al. [5] recommended that eculizumab therapy should be initiated in patients with aHUS—particularly pediatric patients—as early as possible.

The third issue is the susceptibility to infection induced by eculizumab therapy. At the time of writing—17 months after we initiated eculizumab therapy—our patient has remained free of aHUS recurrences despite upper respiratory tract infection and gastrointestinal infection, without any adverse events of this medication. However, the therapy puts all patients at a high risk of neisserial infection because the membrane attack complex plays a crucial role in the elimination of the infection. Therefore, before the administration of eculizumab, the prevention of *Neisseria meningitidis* infection is mandatory. As a general rule, patients receiving eculizumab therapy require a tetravalent meningococcal vaccination. However, the tetravalent vaccines currently available do not cover serogroup B strains, which are the most prevalent *N. meningitidis* strains in Japan, as well as in Europe and America [17]. Moreover, because our patient urgently needed the emergent treatment with eculizumab, he has received continuous antibiotic prophylaxis from the first administration of this medication. The need for prophylactic antibiotics during continuous eculizumab treatment remains controversial. In a prospective and single-arm study involving 41 patients had been vaccinated against *N. meningitidis*, two patients suffered from meningococcal infection during the 26-week study period [15]. Because severe infection by *N. meningitidis* is frequently life-threatening in patients with a late complement component deficiency [18], we recommend antibiotic prophylaxis until the time that quadrovalent conjugate meningococcal vaccines that cover serotype B strains can be launched.

In summary, our patient, who was assumed to have an extremely poor prognosis, responded rapidly to eculizumab therapy, resulting in longitudinal complete remission, improvement of life-threatening hypertension, and cessation of PD. In the near future, eculizumab might be first-line therapy for aHUS if long-term complement blockade can be proved to be safe with meningococcal vaccination. However, the issue of the optimal duration of eculizumab therapy also remains unsettled [5].

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Autoimmune-type atypical hemolytic uremic syndrome treated with eculizumab as first-line therapy

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Abstract We report a case of atypical hemolytic uremic syndrome (aHUS) in a 4-year-old boy. Although the patient had the typical triad of aHUS (microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury), urgent dialysis was not indicated because he had neither oliguria nor severe electrolyte abnormality. He was given eculizumab as first-line therapy, which led to significant clinical improvement, thus avoiding any risk of complications associated with plasma exchange and central venous catheterization. Retrograde functional analysis of the patient's plasma using sheep erythrocytes indicated an increase in hemolysis, suggesting impairment of host cell protection by complement factor H. The use of eculizumab as first-line therapy in place of plasma exchange might be reasonable for pediatric patients with aHUS.

Key words atypical hemolytic uremic syndrome, eculizumab, plasma exchange.

Hemolytic uremic syndrome (HUS) is defined by the typical triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal injury. More than 90% of cases in children are secondary to infection with enterohemorrhagic *Escherichia coli* (EHEC) which produces Shiga toxin. The remaining 10% of cases, however, are classified as atypical hemolytic uremic syndrome (aHUS). aHUS has a poor prognosis with a high mortality rate and a high rate of progression to end-stage renal failure.[1] Plasma exchange (PE) has been recommended as first-line rescue therapy for such aHUS episodes, and for prevention of relapse.[2,3] This treatment, however, has some problems in terms of long-term acceptance, and its efficacy is controversial. Also, vascular access carries risk of complications, including bleeding and vascular injury. Eculizumab (Soliris®; Alexion Pharmaceuticals, Cheshire, CT, USA) is a humanized monoclonal anti-C5 antibody that inhibits the terminal complement pathway and hinders the generation of pro-inflammatory C5a and C5b-9 (membrane attack complex: MAC). Recent reports have indicated the efficacy and safety of eculizumab in patients with aHUS.[4,5] In Japan, it was approved for the treatment of aHUS in September 2013.

Here we describe the clinical features of a child with aHUS due to autoantibody against complement factor H (CFH), who was treated successfully with eculizumab as first-line therapy.

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Case report

The patient was a 4-year-old Japanese boy who was the second child of non-consanguineous parents. He had an elder brother and a younger sister, both of whom were healthy. He had been brought to his family physician with a 2 day history of headache,

nausea, appetite loss, and low-grade fever. Given that anemia, thrombocytopenia, and acute kidney injury were evident, he was tentatively diagnosed as having HUS, and referred to hospital for intensive care. The clinical course is summarized in Figure 1. On admission his complexion was pale and slightly icteric. Other physical data included bodyweight, 14.9 kg; body temperature, 37.5°C; pulse rate, 144 beats/min; and blood pressure, 106/60 mmHg. Neither hepatosplenomegaly nor enlargement of superficial lymph nodes was found. The laboratory findings on admission are summarized in Table 1. Among them, severe anemia, thrombocytopenia, hyperbilirubinemia, elevated lactate dehydrogenase, elevation of serum renal function markers including creatinine, and low C3, were remarkable. Both the direct and indirect Coomb's tests were negative. Hemostatic tests showed that prothrombin time and activated partial thromboplastin time were both within the normal range, but that fibrin/fibrinogen degradation products were elevated. Furthermore, red blood cell (RBC) fragmentation was found in a peripheral blood smear. Stool culture failed to identify Shiga toxin-producing EHEC, or both Shiga toxins 1 and 2. Although the patient had macrohematuria, moderate proteinuria, and elevation of serum renal function markers, he did not fall into the category of oliguria or severe electrolyte abnormality. For this reason, urgent dialysis was not initiated. On the following day (hospital day [HD] 2), fresh frozen plasma (FFP; 23 mL/kg) was infused in order to supply normal complement regulatory factors under a tentative diagnosis of aHUS, given that diarrhea was absent. On the third day (HD 3), however, the patient's clinical symptoms worsened, and RBC concentrates were therefore transfused. Given that plasma a disintegrin-like and metalloproteinase with thrombospondin type I motifs, number 13 (ADAMTS13) activity was 120% on the night of HD 3, the patient was definitively diagnosed as having aHUS, and given eculizumab at a dose of 600 mg. On the second day after eculizumab treatment (HD 5), the macrohematuria dramatically resolved, and thereafter hematology showed gradual improvement. On HD 10, the patient started to receive eculizumab at the maintenance dose (300 mg) by injection every 2 weeks. The patient was vaccinated against *Neisseria meningococcus* and *Streptococcus pneumonia* on HD 29 and HD37, respectively, and received prophylactic antibiotic therapy with cefditoren pivoxil until 2 weeks after vaccination for meningococcus. He was discharged with no sequelae on HD32, and thereafter received an injection of eculizumab at the maintenance dose (300 mg) every 2 weeks. There were no adverse events associated with eculizumab treatment, including infusion reaction or infection, in the whole period of observation, or any further recurrence of aHUS.

Retrograde analysis including hemolytic assay, and Western blotting for detection of anti-CFH antibody and complement factor H-related protein 1/3 (CFHR 1/3) were performed using the patient's plasma, which had been obtained before plasma infusion using the method reported previously.[6,7] Comprehensive gene mutation analysis of CFH, complement factor I (CFI), complement factor B (CFB), C3, membrane cofactor protein (MCP), and thrombomodulin, was also performed as described previously.[6] The patient's plasma enhanced the hemolysis of sheep erythrocytes and this effect was suppressed by addition of purified CFH, indicating impairment of host cell protection by CFH (Fig. 2). Anti-CFH antibody was detected in the patient's plasma, but no deficiency of the protein encoded by CFHR 1/3 was observed (Fig. 2).[8] Additionally, there were no mutations of CFH, CFI, CFB, C3, MCP, or thrombomodulin. Therefore, the patient was diagnosed as having aHUS due to autoantibody against CFH without CFHR 1/3 protein deficiency.

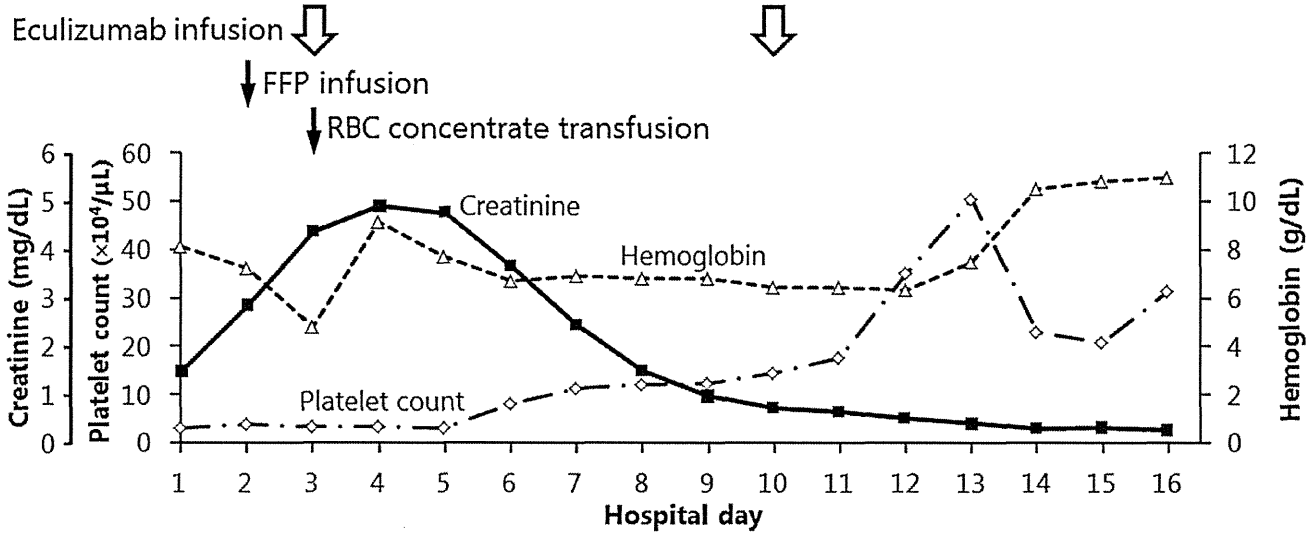

Fig. 1 Clinical course of treatment for atypical hemolytic uremic syndrome. Although fresh frozen plasma (FFP; 23 mL/kg) and red blood cell (RBC) concentrate were transfused on hospital days 2 and 3, laboratory findings including creatinine and platelet count worsened. After initiation of eculizumab, however, all of the laboratory parameters improved.

Table 1 Laboratory findings on admission

Peripheral blood		Stool culture	
WBC	9500 /μl	Normal flora	
RBC	300×10^4 /μl	EHEC	(−)
Hb	8.1 g/dL	Shiga-toxin	(−)
Ht	23.2%		
Platelet	1×10^4 /μl	Chemistry	
RBC fragmentation	(+)	TP	6.1 g/dL
		Alb	3.1 g/dL
Hemostatic test		Total bilirubin	2.6 mg/dL
PT	11.2 s	Indirect bilirubin	2.2 mg/dL
PT-INR	1.06	AST	101 IU/L
APTT	27.1 s	ALT	27 IU/L
Fibrinogen	278 mg/dL	LDH	3,570 IU/L
FDP	11.8 μg/mL	BUN	48.7 mg/dL
		UA	9.3 mg/dL
Urinalysis		Cr	1.51 mg/dL
Urine color	Light red	CRP	3.55 mg/dL
Occult blood	(4+)	Na	136 mmol/L
Protein	(2+)	K	3.8 mmol/L
Sediment		Cl	102 mmol/L
RBC	10–15/HPF	CK	403 IU/L
WBC	1–4/HPF	Haptoglobin	<10 mg/dL
Epithelium		Coombs test	
Epithelial cast	1+	Direct	(−)
		Indirect	(−)
Complement activity			
CH50	32.2 IU/L	Serological test	
C3	30.8 mg/dL	Total ANA	<40
C4	21.8 mg/dL	PR3-ANCA	<1.0
		MPO-ANCA	<1.0
ADAMTS13 activity	120%	ss-DNA antibody	<1.0
		ds-DNA antibody	<1.0

ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13; ANCA, anti-neurophil cytoplasmic antibody; APTT, activated partial thromboplastin time; EHEC, enterohemorrhagic *Escherichia coli*; FDP, fibrin/fibrinogen degradation products; INR, international normalized ratio; MPO, myeloperoxidase; PR3, proteinase 3; PT, prothrombin time.

Discussion

Atypical hemolytic uremic syndrome is a rare disease characterized by hemolytic anemia, thrombocytopenia, and acute renal failure secondary to thrombotic microangiopathy. In recent years, aHUS has been found to be associated with dysregulation of the complement alternative pathway. In more than half of patients with aHUS, mutations in genes encoding complement-regulating protein including CFH, CFI, and MCP, have been reported.[1] Additionally, functional CFH deficiency due to autoantibodies against CFH has been reported, and this is highly associated with polymorphic homozygous deletion of genes encoding CFHR proteins 1 and 3.[1] The present patient had had no diarrhea, and neither EHEC nor Shiga toxin had been found in his stools. ADAMTS13 activity was 120%, which was within the normal range. aHUS associated with anti-CFH autoantibody was diagnosed on the basis of additional examinations including gene mutation analysis and Western blot analysis for anti-CFH antibody and proteins encoded by CFHR1/3.

Plasma exchange has been recommended as a first-line therapy for aHUS based on expert opinion rather than clinical trials.[2,3] For management of aHUS associated with anti-CFH autoantibodies, PE with FFP has been done for the purpose of removing anti-CFH autoantibodies and simultaneously supplying the circulating CFH pool. Although combination therapy with immunosuppressants has also been used, the rate of remission in response to short-term PE is 70–80%, and the rate of death or end-stage renal disease as a long-term outcome is 30–40% in patients with anti-CFH autoantibodies.[1] Additionally, it is difficult to determine whether the disease activity is stable and leads to remission, because no international standard for determining anti-CFH antibody and the levels of autoantibodies leading to disease relapse or exacerbation has been established.

In contrast, previous case reports have suggested that eculizumab is effective for treatment of aHUS.[4,5] Additionally, Legendre *et al.* noted the efficacy and safety of long-term eculizumab for thrombotic microangiopathy in aHUS patients, via two prospective phase 2 trials lasting 62–64 weeks.[9] Although reduction of the antibody load plays a very important role in aHUS associated with anti-CFH autoantibodies, eculizumab can effectively block the terminal complement cascade and stop further damage in the presence of anti-CFH autoantibodies. Noone *et al.* reported two cases of CFH autoantibody-positive HUS treated with eculizumab and proposed that eculizumab should be used in the acute phase for arresting the complement-mediated damage.[10]

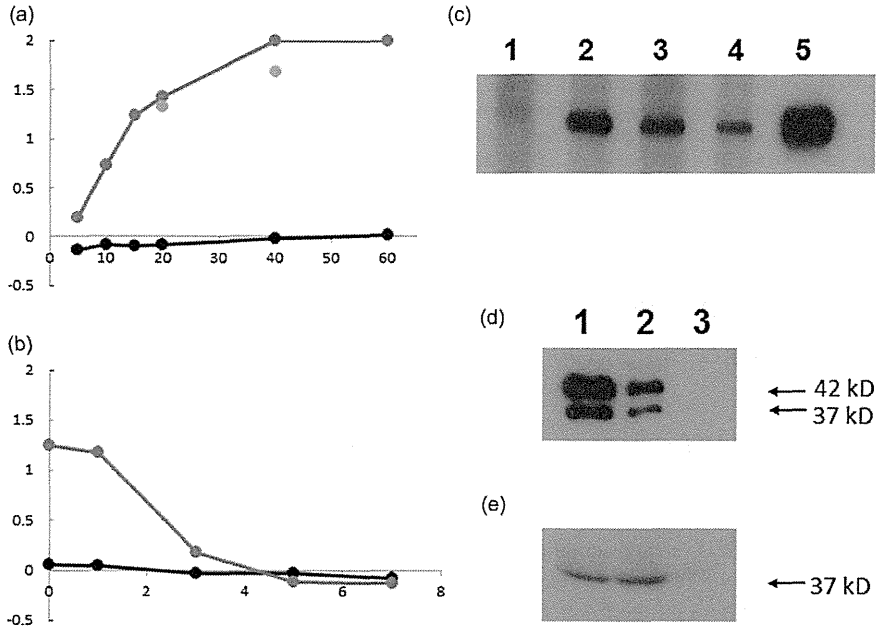

Fig. 2 (a,b) Hemolytic test and (c–e) Western blot analysis for detection of (c) anti-complement factor H (anti-CFH) autoantibodies and (d,e) protein encoded by *complement factor-H related protein CFHR1* and 3. (a) Lysis of sheep erythrocytes by addition of patient plasma. (a) OD_{414} titer sheep erythrocytolysis as a function of patient plasma. Plasma samples ranging from 5 μL to 60 μL were used. (b) Inhibition of enhanced hemolysis with 20 μL of plasma by adding purified CFH in amounts ranging from 0 μg to 7 μg. (—●—) Normal plasma ; (—●—) patient plasma; (●) normal plasma plus purified anti-CFH antibody. (c) Western blot analysis for detection of anti-CFH autoantibody. Lane 1, normal plasma; lane 2, patient plasma in the acute phase (hospital day [HD] 2); lane 3, patient plasma in the chronic phase (HD 31); lane 4, plasma of 3-year-old Japanese boy diagnosed with aHUS associated with anti-CFH autoantibody;[8] lane 5, CFH monoclonal antibody. (d,e) Western blot analysis for (d) CFHR 1 and (e) 3 proteins. (d) Plasma samples from a normal control, the present patient, and a patient who had been previously diagnosed as having deficiency of CFHR plasma proteins and autoantibody-positive HUS (DEAP-HUS)[8] were electrophoresed on a 12.0% SDS-polyacrylamide gel and transferred to a polyvinylidene fluoride membrane. After blocking with 5% dried milk, the membrane was incubated for 1.5 h at room temperature with mouse anti-human CFHR1 monoclonal antibody, the concentration of which was adjusted to 1 μg/mL. Then, 10 000-fold-diluted horseradish peroxidase (HRP)-labeled goat anti-mouse IgG antibody was used as the secondary antibody, and bound mouse monoclonal antibody was visualized using enhanced chemiluminescence substrate (Western Lightning-ECL; Perkin Elmer, Yokohama, Japan). (e) Western blot analysis for detection of CFHR 3 protein was done using the same method as for CFHR 1, with 1500-diluted rabbit anti-human CFHR 3 polyclonal antibody as the first antibody and 20 000-diluted HRP-labeled goat anti-rabbit IgG antibody as the secondary antibody. Lane 1, normal control; lane 2, present patient; lane 3, DEAP-HUS patient.[8]

Given that the present patient had neither oliguria nor electrolyte abnormalities including hyperkalemia, urgent dialysis was not necessary. Therefore, the patient received eculizumab as first-line therapy and was able to avoid the risk of complications associated with these maneuvers. Therapy with eculizumab was very effective, and no adverse events occurred. Zuber *et al.* proposed the use of eculizumab as first-line therapy for all episodes of aHUS in children because of its efficacy and safety, and for avoiding any potential complications of PE.[4]

Conclusion

The present study has demonstrated the efficacy and short-term safety of eculizumab as first-line therapy in the acute phase for aHUS associated with anti-CFH autoantibodies in a pediatric patient.

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