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Case Report

Treatment of Congenital Thrombotic Thrombocytopenic Purpura With Eculizumab

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A 12-year-old boy was hospitalized for hemolytic anemia, thrombocytopenia, acute kidney injury, and generalized seizures. The childhood onset, severely decreased kidney function, absence of prodromal diarrhea, negative test results for Shiga-like toxin-producing *Escherichia coli*, elevated plasma levels of the terminal complement complex sC5b-9, and ex vivo testing in endothelial cells showing serum-induced complement activation were all consistent with a diagnosis of complement-mediated atypical hemolytic uremic syndrome. Before plasma ADAMTS13 (von Willebrand factor protease) activity results were available, the patient was treated with the anti-C5 monoclonal antibody eculizumab, and treatment was followed by prompt disease remission. However, results of ADAMT13 activity level tests and gene screening revealed a severe deficiency associated with 2 heterozygous mutations in the *ADAMTS13* gene, fully consistent with a diagnosis of congenital thrombotic thrombocytopenic purpura. Screening for atypical hemolytic uremic syndrome–associated genes failed to show a mutation and an assay for plasma anti–factor H antibodies gave negative results both before and after eculizumab treatment initiation. The patient's clinical evolution suggests that complement activation plays a role in the pathogenesis of thrombotic thrombocytopenic purpura and provides unexpected new insights into the treatment of this life-threatening disease.

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INDEX WORDS: Thrombotic thrombocytopenic purpura (TTP); congenital TTP; ADAMTS13; von Willebrand factor (vWF) protease; ultralarge vWF (ULvWF); complement; eculizumab; terminal complement pathway; atypical hemolytic uremic syndrome (aHUS); thrombotic microangiopathy (TMA).

Thrombotic thrombocytopenic purpura (TTP) is a rare disease that features thrombocytopenia, microangiopathic hemolytic anemia, and widespread microvascular thrombi that result in multiorgan dysfunction.[1] Neurologic injury is common and has historically been used to differentiate TTP from atypical hemolytic uremic syndrome (aHUS), a related thrombotic microangiopathy in which acute kidney injury is a prominent feature.[2] However, acute and chronic kidney disease may be seen in patients with TTP,[3] and aHUS can involve extrarenal manifestations, so it can be difficult to discern the 2 diseases solely by clinical presentation.

TTP is associated with a deficiency in ADAMTS13, a plasma metalloprotease that cleaves von Willebrand factor (vWF) multimers, with the consequent appearance of ultralarge vWF (ULvWF) multimers in the blood circulation.[4] ADAMTS13 deficiency in TTP is generally due to autoantibodies that typically are no longer detectable during remission. In 5% to 10% of cases, the enzymatic deficiency is congenital and caused by mutations in the *ADAMTS13* gene. The mainstay of therapy in congenital TTP is fresh frozen plasma infusions or plasma exchange to supply enough ADAMTS13 protein to cleave the ULvWF multimers.[5] In patients with recurrent congenital TTP, prophylactic fresh frozen plasma is often administered every 2 to 3 weeks to maintain ADAMTS13 levels high enough to cleave the ULvWF multimers and prevent the formation of microthrombi.[5] However, plasma treatment is associated with morbidity and mortality, including the acute risk for allergic reactions/anaphylaxis and transfusion-related acute lung injury and the long-term risk for infection (historically with hepatitis B virus, hepatitis C virus, and HIV and more recently with the prion-associated Creutzfeldt-Jacob disease, which is resistant to current inactivation procedures).[6]

Unlike TTP, aHUS is associated with genetic or acquired disorders of components of the complement alternative pathway, which predispose to complement-mediated endothelial injury.[2] The anti-C5 monoclonal antibody eculizumab blocks C5

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cleavage, preventing terminal complement pathway activation and protecting from microvascular thrombosis, radically improving the outcome of patients with aHUS.[7]

In this report, we describe a child who originally had aHUS diagnosed and who was treated with eculizumab. Treatment was promptly followed by remission. However, he was later found to have severe ADAMTS13 deficiency, associated with 2 *ADAMTS13* mutations, a finding fully consistent with a diagnosis of congenital TTP. This case supports the emerging hypothesis that complement is activated in the setting of ADAMTS13 deficiency[8] and may contribute to microvascular thrombosis in patients with congenital TTP.

CASE REPORT

A 12-year-old Italian boy presented to the Pediatric Nephrology Unit of Santobono-Pausilipon Hospital in early 2012 with Coombs-negative hemolytic anemia (hemoglobin, 7.8 g/dL; lactate dehydrogenase, 1,449 IU/L; undetectable haptoglobin, and schistocytes in the blood smear), thrombocytopenia (platelet count, 9×10^3/μL), acute kidney injury requiring hemodialysis (estimated glomerular filtration rate [eGFR], 7 mL/min/1.73 m^2 as calculated using the bedside Schwartz equation[9]), and generalized seizures, preceded by an upper respiratory tract infection treated with antibiotics. Full laboratory values are provided in Table 1, and a timeline of the events immediately preceding and following admission is shown in Fig S1 (available as online supplementary material).

The childhood onset, severely decreased kidney function, absence of prodromal diarrhea, negative test results (from stool culture and serology) for Shiga-like toxin-producing *Escherichia coli*, and the elevated plasma level of the terminal complement complex sC5b-9 (520 [reference range, <400] ng/mL], measured using the MicroVue SC5b-9 Enzyme Immunoassay [Quidel]) were all consistent with a diagnosis of complement-mediated aHUS.[2] In order to rule out TTP, regulatory authority recommendations are to assay plasma ADAMTS13 activity before initiating eculuzumab treatment.[10] However, given the severity of the patient's clinical condition, the clinician decided to start eculizumab (900 mg intravenous, 4 doses weekly; then 1,200 mg approximately every 2 weeks; Fig 1) 4 days after admission and before the ADAMTS13 test results were available (antimeningococcal vaccination and antibiotic prophylaxis were administered before eculizumab initiation). The response was excellent (Figs 1, S2-S4); within 3 days, platelet count, lactate dehydrogenase level, and diuresis normalized; dialysis therapy was discontinued; serum creatinine level decreased to 1.78 mg/dL (eGFR, 36 mL/min/1.73 m^2); and the severity of the patient's anemia lessened (hemoglobin, 8.9 g/dL).

Table 1. Laboratory Parameters at Admission

Parameter	Value	Reference Range
Sodium, mEq/L	120	134-145
Potassium, mEq/L	5.8	3.5-5
Calcium, mg/dL	8.9	8.2-10.2
Phosphorus, mg/dL	8.2	2.5-4.5
Creatinine, mg/dL	9.31	0.5-1.2
eGFR, mL/min/1.73 m^2	7	80-120
Serum urea nitrogen, mg/dL	364	10-20
White blood cell count, $\times 10^3$/μL	8.3	4.5-11
Red blood cell count, $\times 10^6$/μL	3.6	4.5-6
Hemoglobin, g/dL	7.8	14-18
Platelets, $\times 10^3$/μL	9	150-400
Lactate dehydrogenase, IU/L	1,449	120-290
Haptoglobin, g/L	<0.08	0.4-0.8

Abbreviation: eGFR, estimated glomerular filtration rate.

After the sixth eculizumab dose, an attempt to space out subsequent infusions resulted in thrombocytopenia (platelet count of 11×10^3/μL, with diffuse petechial lesions) and microangiopathic hemolysis, with normal kidney function (eGFR, 115 mL/min/1.73 m^2). A recently published[11] ex vivo test for endothelium-restricted complement activation in aHUS (methodology provided in Item S1) showed that serum taken from the patient during the relapse deposited high amounts of C5b-9 on endothelial cells, indicating a role of complement in disease relapse (Fig 1).[11] Thus, eculizumab (1,200 mg) was reintroduced, promptly resolving the thrombocytopenia within 24 hours (Fig 1); the petechial lesions disappeared within 48 hours. After eculizumab treatment, ex vivo testing of the patient's serum no longer showed elevated C5b-9 deposits. The patient continued receiving eculizumab biweekly until day 140, at which point interdose intervals were lengthened until discontinuation. In the subsequent year, he had 5 hematologic relapses (without renal or neurologic symptoms), often associated with upper respiratory tract infections; each was effectively treated with a single dose of eculizumab. After tonsillectomy in summer 2013, no further relapse occurred during a further 22-month drug-free follow-up.

While the boy was already receiving treatment, screening of aHUS-associated genes (*CFH* [complement factor H], *CD46* [encoding membrane cofactor protein], *CFI*, *CFB*, *C3,* and *THBD* [encoding thrombomodulin]) was performed using next-generation sequencing on an Ion Torrent Personal Genome Machine (Life Technologies). This failed to show any mutation. However, the presence of genetic abnormalities in other complement-related genes cannot be ruled out.

Both before and after eculizumab initiation, anti-CFH antibodies were undetectable by plasma enzyme-linked immunosorbent assay (performed as described in[12]). During the acute phase and also in remission, measurement of ADAMTS13 activity (in citrated plasma) showed undetectable levels (<6% using a collagen-binding assay and <3% by fluorescence resonance energy transfer [FRET] using the ADAMTS13 fluorogenic substrate FRETS-rVWF73),[13] without evidence of inhibitory autoantibodies. By sequencing *ADAMTS13*, we found 2 heterozygous mutations (a guanine to adenine change at nucleotide 3,251 of the complementary DNA, which is predicted to cause a cysteine to tyrosine substitution at amino acid 1,084, and has been previously reported in patients with TTP,[14-16] and a previously unpublished frameshift [from deletion of the cytosine at nucleotide 4,049 of the complementary DNA] after the arginine at amino acid 1,351, which is predicted to lead to a premature stop codon 9 amino acids later, Fig S5). Taken together, screening results were consistent with a diagnosis of congenital TTP.

DISCUSSION

We report what to our knowledge is the first case of congenital TTP treated with the complement inhibitor eculizumab. The prompt disease remission after eculizumab treatment, both at the onset and during recurrences, supports the recent idea that the alternative complement pathway is activated in the presence of ADAMTS13 deficiency[8] and suggests that complement plays a pathogenetic role in microvascular thrombosis.