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BRIEF COMMUNICATION

Genetic predisposition to infection in a case of atypical hemolytic uremic syndrome

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Abstract

Most cases of hemolytic uremic syndrome (HUS) are caused by infection with enterohemorrhagic *Escherichia coli* (EHEC). Genetic defects causing uncontrolled complement activation are associated with the more severe atypical HUS (aHUS). Non-EHEC infections can trigger the disease, however, complement defects predisposing to such infections have not yet been studied. We describe a 2-month-old patient infected with different Gram-negative bacterial species resulting in aHUS. Serum analysis revealed slow complement activation kinetics. Rare variant R229C was found in complement inhibitor vitronectin. Recombinant mutated vitronectin showed enhanced complement inhibition in vitro and may have been a predisposing factor for infection. Our work indicates that genetic changes in aHUS can not only result in uncontrolled complement activation but also increase vulnerability to infections contributing to aHUS.

Introduction

Hemolytic uremic syndrome (HUS) is a devastating renal disease, which is characterized by hemolytic anemia, thrombocytopenia, and acute renal failure. Most HUS cases are caused by infection with enterohemorrhagic *Escherichia coli* (EHEC). However, 5–10% of HUS patients have a more severe atypical form (aHUS). Overactive complement is considered to be a central element in aHUS pathogenesis [1].

The complement system, a part of the innate immune system, can be activated via three pathways: the classical, the lectin, and the alternative. These pathways converge at the activation of the complement component C3 further leading to formation of the terminal C5b-9 complement complex (TCC) [1].

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Sequence variants that lead to impaired complement regulation of C3 activity in aHUS are found in genes encoding CFH, CFI, MCP, C3, CFB and thrombomodulin. Moreover, important C3 convertase inhibitor CFH can be affected by autoantibodies (anti-CFH) [1]. Next to these abnormalities, aHUS episodes can sometimes be triggered by non-EHEC infections, including *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, influenza A, HIV and others [1, 2]. Several cases of infection with *Bordetella pertussis* have also been reported [3–5]. The infections trigger initial complement attack, which cannot be adequately controlled due to dysregulating genetic changes and may cause renal damage in aHUS. Nevertheless, genetic predisposition to infections in aHUS has not yet been studied.

Table 1 Complement activity assessment in aHUS patient

Complement pathway	Patient's value	Reference range
Classical pathway (%)[a]	77.4	69–129
Alternative pathway (%)[a]	75.1	30–113
Lectin pathway (MBL-mediated) (%)[a]	18.2	0–125
Plasma vitronectin concentration (μg/mL)[b]	190	185–595[c] (286, $n = 20$)[d]

[a]Measured using Wieslab® Complement system Screenkit (Euro Diagnostica) and given as percentage of the positive control, provided with the assay. Reference range is presented as indicated by the manufacturer

[b]Measured using Human Vitronectin Total ELISA Kit (Innovative Research)

[c]Reference range was determined as range of values, measured in a healthy control group

[d]Median value and number of analyzed controls are indicated in parenthesis

Case report

A female infant was diagnosed with whooping cough at the age of 7 weeks. The presence of *Bordetella pertussis* infection was confirmed by serological analysis, where IgG values increased from 1 Virotech unit (VE)/mL to 10 VE/mL in first 8 days of the disease. At day 10 after the onset of infection, the patient developed acute renal failure (serum creatinine 257 μmol/L, urea 27.5 mmol/L), hemolytic anemia (Hb 2.8 mmol/L, LDH 3591 U/L), and mild thrombocytopenia (platelets 147×10^9/L) and was consequently diagnosed with aHUS. Infection with EHEC O157 was excluded by fecal culture and PCR. ADAMTS13 activity was normal (53%), which excluded thrombotic thrombocytopenic purpura. At this time the patient also developed pneumonia (sputum positive for *Moraxella catarrhalis*) and sepsis (*Klebsiella oxytoca* in blood). The infant received blood transfusions, continuous veno-venous hemofiltration

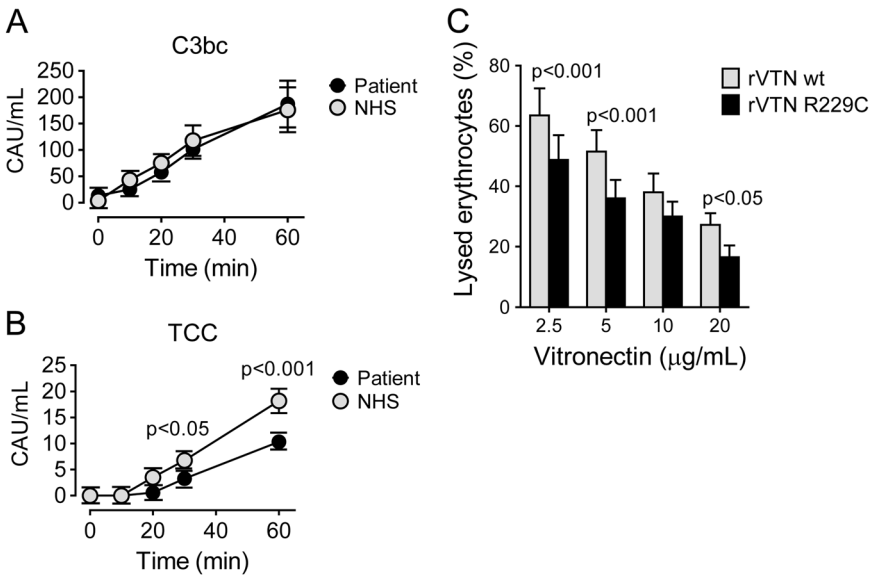

Fig. 1 **a** Serum of patient carrying R229C mutation in vitronectin and normal human serum (NHS) pool were incubated at 37 °C with gentle agitation. Samples were collected at 0, 10, 20, 30, and 60 min of incubation and C3bc levels were quantified in complement activation units per mL (CAU/mL) using international complement standard #2 [9]. Data were collected in three independent experiments and presented as mean ± standard error. **b** TCC levels were quantified in the same experimental set up as C3bc [9]. **c** TCC was allowed to form on the surface of sheep erythrocytes from purified components of (C5b6, C7, C8, C9) as previously described [10] in the presence of various concentrations of purified recombinant vitronectin variants. The wild type (rVTN wt) and mutant (rVTN R229C) vitronectin variants (amino acids 20–396) were produced in HEK293T cells as previously described [11]. Efficiency of TCC formation was quantified as percentages of lysed erythrocytes. Data were collected in four independent experiments and presented as mean + standard error

(CVVH) and was ventilated. She recovered completely from the aHUS 10 days later (20 days after the onset of the pertussis) with normalized renal function (serum creatinine 43 µmol/L, urea 1 mmol/L). Eculizumab treatment was not yet available at that time. During a 10-year follow-up by pediatric nephrologist, patient presented no other episode of aHUS or severe infection.

Due to infections with Gram-negative bacteria, we analyzed activity of the three complement pathways after the patient had recovered from aHUS. These were within the normal range (Table 1). The in vitro complement activation of patient serum was compared to that of normal human serum (NHS). In patient's serum, C3 activation rate (expressed as generation of C3bc) was comparable to the rate in NHS, but the TCC generation was delayed (Fig. 1a, b).

Genetic screening of the alternative pathway indicated presence of a heterozygous missense variant in thrombomodulin (A43T), which was previously described as pathogenic in aHUS [6]. No other changes in alternative pathway or anti-CFH autoantibodies were detected.

Because kinetic experiments have shown decreased rate of TCC generation, we analyzed the patient for possible defects in genes encoding TCC components and TCC inhibitors (vitronectin, clusterin, and CD59). A heterozygous variant rs782409757:c.685C > T (R229C) was found in gene encoding vitronectin. In silico analysis indicated this variant as deleterious (SIFT score 0.0) and probably damaging (PolyPhen-2 score 1.0), as a large positively charged amino acid is replaced by an unpaired cysteine. Patient vitronectin plasma levels were normal (Table 1). In vitro experiments using purified recombinant proteins revealed that mutant vitronectin was more potent in complement inhibition compared to the wild type (Fig. 1c).

The c.685C>T (R229C) is a rare variant, reported with the frequency of 0.0015% in European population (http://exac.broadinstitute.org/variant/17-26696034-G-A). Moreover, we tested the variant in 390 Dutch children who survived bacterial meningitis, since variation in innate immune response genes also affects susceptibility to meningitis [7]. None of the children had the vitronectin variant, which may be explained by low incidence of the change.

Discussion

The aHUS cases associated with *Bordetella pertussis* have been described before, however, to our knowledge, this is the first case associated with simultaneous isolation of *Klebsiella oxytoca* and *Moraxella catarrhalis*. Although exact contribution of individual pathogens is not clear, each additional infection may have contributed to more systemic inflammation, leading to aHUS.

Functional assessment of the complement system did not reveal major abnormalities (Table 1). Low functional activity of MBL-mediated lectin pathway was found, which is very common in the human population thus not considered as defect. In our previous work, we have found that MBL deficiency is not more common in aHUS patients than in healthy controls [8].

The rate of TCC formation in patient's serum was slower than in the NHS. Consistently, TCC assembled from purified components in the presence of recombinant vitronectin with R229C was less functional. Although other factors may have delayed TCC formation in vivo, our studies using recombinant vitronectin demonstrate that the R229C change certainly played a role. Delayed TCC response may have caused inefficient initial clearance of Gram-negative bacteria in the patient, which resulted in infection and profound complement activation. Due to the presence of thrombomodulin change, complement attack was poorly controlled at the level of C3 activation with aHUS episode as a result.

Importantly, aHUS patients are currently treated with TCC blocker eculizumab. Patients that carry TCC inhibiting variants, as for the first time described here, may require lower drug doses.

Taken together, our work indicates that a genetic cause may contribute to aHUS not only by a well-known effect of complement dysregulation, but also by enhancing vulnerability to infections during early infancy.

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Compliance with ethical standards

Conflict of interest Dr. N.C.A.J. van de Kar is a member of the international advisory board of Alexion. The remaining authors declare no conflict of interest.

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