

# Anti-Factor H Autoantibodies Associated with Atypical Hemolytic Uremic Syndrome

Marie-Agnès Dragon-Durey,\* Chantal Loirat,<sup>†</sup> Sylvie Cloarec,<sup>‡</sup> Marie-Alice Macher,<sup>†</sup> Jacques Blouin,\* Hubert Nivet,<sup>‡</sup> Laurence Weiss,<sup>§</sup> Wolf Herman Fridman,<sup>\*||</sup> and Véronique Frémeaux-Bacchi,<sup>\*||</sup>

\*Service d'Immunologie Biologique, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris;

<sup>†</sup>Service de Néphrologie, Hôpital Robert Debré, Assistance Publique-Hôpitaux de Paris, Paris; <sup>‡</sup>Unité de Néphrologie Pédiatrique, Centre Hospitalier de Tours, Tours;

<sup>§</sup>Service d'Immunologie Clinique, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris, Paris; and <sup>||</sup>INSERM U255, Institut des Cordeliers, Paris, France

Several studies have demonstrated genetic predisposition in non-shiga toxin-associated hemolytic uremic syndrome (HUS), involving regulatory proteins of the complement alternative pathway: Factor H (FH) and membrane co-factor protein (CD46). Regarding the observations of thrombotic thrombocytopenic purpura patients, in whom a von Willebrand factor protease (ADAMST-13) deficiency may be inherited or acquired secondary to IgG antibodies, it was speculated that HUS might occur in a context of an autoimmune disease with the development of anti-FH antibodies leading to an acquired FH deficiency. The presence of FH autoantibodies was investigated by an ELISA method using coated purified human FH in a series of 48 children who presented with atypical HUS and were recruited from French university hospitals. Anti-FH IgG antibodies were detected in the plasma of three children who presented with recurrent HUS. The anti-FH specificity was conserved by the Fab'2 fraction. The plasma FH activity was found to be decreased, whereas plasma FH antigenic levels and FH gene analysis were normal, indicating that the presence of anti-FH antibodies led to an acquired functional FH deficiency. This report supports for the first time that HUS may occur in a context of an autoimmune disease with the development of anti-FH-specific antibody leading to an acquired FH deficiency. This new mechanism of functional FH deficiency may lead to the design of new approaches of diagnosis and treatment with a particular interest in plasma exchanges or immunosuppressive therapies.

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**T**hrombotic microangiopathy (TMA) disorders include thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) (1). The common clinical features of TMA associate microangiopathic hemolytic anemia, thrombocytopenia, and variable organ damage. Classically, HUS is characterized by an acute renal failure, whereas in TTP, neurologic symptoms are predominant. Typical HUS occurs in infants and young children, in relation to shiga toxin (Stx) produced by some strains of bacteria such as 0157/H7 *Escherichia coli* and has a good outcome. In contrast, atypical HUS occurs at any age, including newborn, and has a frequently recurrent course and a poor renal prognosis. Several studies have demonstrated genetic predisposition in atypical HUS, in both familial and sporadic cases, involving two regulatory proteins of the complement alternative pathway: Factor H (FH) and membrane co-factor protein (MCP; or CD46). The human complement system can be activated through three

different pathways: The classical and lectin pathways and the alternative pathway that converge to generate either classical or alternative C3-convertase enzymes. C3 convertase enzymes' function is the proteolytic activation of the third component of complement C3 to C3a and C3b, leading to covalent attachment of C3b to surfaces during complement activation. FH and MCP serve as co-factors for the C3b-inactivating enzyme complement factor I. FH also inhibits the formation and accelerates the decay of the alternative C3-convertase. FH is a single-chain serum glycoprotein of 150 kD with modular structure consisting of a tandem array of 20 homologous units (2), called short consensus repeats (SCR) or complement control protein, composed of approximately 60 amino acid residues each (3). Homozygous or heterozygous FH deficiencies (reviewed in 4) as well as single amino acid exchanges within the C-terminal domains of the protein have been reported to be associated with atypical forms of HUS (5-9). Richards *et al.* (10) and Noris *et al.* (11) found mutations in the CD46 gene in four affected families. However, >50% of cases, including HUS patients with decreased plasma C3 concentrations, remain without identified etiologic factor. Regarding these observations, we speculate that HUS might occur in a context of an autoimmune disease as described in patients with TTP, in whom a von Willebrand factor protease (ADAMST-13) deficiency may be inherited or

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**Address correspondence to:** Dr. Marie-Agnès Dragon-Durey, Service d'Immunologie Biologique, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75 015 Paris, France. Phone: 33-1-56-09-39-41; Fax: 33-1-56-09-20-80; E-mail: [marie-agnes.durey@egp.ap-hop-paris.fr](mailto:marie-agnes.durey@egp.ap-hop-paris.fr)

acquired secondary to IgG antibodies (12–16). We investigated for the presence of FH autoantibodies in a series of 48 children who presented with atypical HUS. We report for the first time on three children who developed anti-FH autoantibodies in a context of atypical HUS.

## Materials and Methods

### Patients

Forty-eight children who presented with atypical (non-Stx-related) HUS were recruited from the departments of pediatric nephrology of French University hospitals from 1997 to 2003. EDTA plasma samples were obtained from patients and stored at  $-70^{\circ}\text{C}$ . Informed consent of parents of children was obtained before DNA analysis.

### Complement Assays

Measurement of CH50 activity in EDTA plasma samples was performed as described previously (17). Plasma concentrations of the complement components C4, C3, and factor B (FB) antigens were measured by nephelometry (Dade Behring, Paris La Defense, France). FH antigen concentrations were measured by sensitive ELISA methods as described previously (4).

FH activity was measured by assessing the ability of test plasma to dissociate a preformed cell-bound C3bBb convertase, as described previously (18). Results were expressed as the percentage of values obtained in the same experiment with a reference plasma pool prepared from 100 healthy blood donors (normal ranges were  $100 \pm 30\%$  as calculated with the results from 50 individual healthy donors).

### Genomic FH DNA Sequencing

For genomic FH DNA analysis, genomic DNA was extracted from whole blood using the proteinase K/phenol method (19). Uncloned genomic DNA was amplified by means of a PCR using oligonucleotides flanking each exon. Primer sequences, length of the PCR-amplified fragments, and temperatures of hybridization used for each reaction and direct DNA sequencing procedure have been previously described (4). Sequence analyses were performed using the Sequencher software.

### Anti-FH Antibody Assessment

Presence of anti-FH antibody was detected by using an ELISA method. Nunc MaxiSorp ELISA plates (Nunc, Roskilde, Denmark) were coated with  $0.3 \mu\text{g}$  of purified human FH (Calbiochem, Meudon, France). After washing and blocking free reactive sites with PBS that contained 1% BSA, the plasma to be tested was added at a dilution of 1:50 for 1 h at room temperature. After washing, the plates were incubated for 1 h at room temperature with a goat anti-human IgG antibody, specific for the  $\gamma$  chain, labeled with horseradish peroxidase (Sigma-Aldrich, Steinheim, Germany). After additional washing, enzymatic activity was revealed using the orthophenyldiamine substrate. Optical density above the mean  $+ 2$  SD of those obtained in 50 individual healthy donors' plasma was considered as positive.

Positive samples were subsequently tested at serial dilutions, and a titer was determined. Antibody titers were expressed as arbitrary units per ml (AU/ml) calculated as  $(S - B) \times 1000 / (P - B)$ , where S is optical density of the test sample diluted at 1/200, B is absorbency of the blank, and P is absorbency of the reference positive plasma diluted (1:200) given an arbitrary titer of 1000 AU/ml.

The isotype of the IgG anti-FH antibodies was determined by the use of mouse monoclonal anti-IgG1, IgG2, and IgG3 antibody at the second step of the ELISA method. After additional washing, plates were incubated with an anti-mouse IgG labeled with horseradish peroxidase (Sanofi Pasteur, Marne-la-Coquette, France) and further incubated for

1 h at room temperature. Enzymatic activity was revealed using the orthophenyldiamine substrate.

### Purification of IgG and Fab'2 Preparation

Total IgG was purified from plasma of two positive patients (patients 1 and 2) on protein G Sepharose followed by acid elution. The eluted IgG-containing fractions were then dialyzed against PBS. The purity of IgG preparations was assessed by SDS-PAGE under reducing and nonreducing conditions.

For Fab'2 preparation, the purified IgG were dialyzed against sodium acetate ( $\text{CH}_3\text{COONa}$ ) 0.1 M (pH = 4.1) at room temperature during 90 min. Twenty micrograms of Pepsine (3045 units/mg protein; Sigma-Aldrich) were added for 1 mg of IgG and incubated during 18 h at  $37^{\circ}\text{C}$ . Enzymatic reaction was stopped by adjusting pH to 7 with a TRIS 2 M solution. The Fab'2 preparations were then dialyzed against PBS. The purity of Fab'2 preparations was assessed by SDS-PAGE under reducing and nonreducing conditions.

### FH Binding Inhibiting Assays

Inhibition of the IgG binding to immobilized FH by fluid-phase FH was performed. Diluted (1:500) plasma was preincubated with increasing amounts (0 to  $10 \mu\text{g}$ ) of purified FH (Calbiochem, Meudon, France) during 18 h at  $4^{\circ}\text{C}$  and then directly tested in an FH-coated microtiter plate, and ELISA was performed as described previously. Positive plasma was also tested by ELISA with co-incubation of serial dilutions of sheep polyclonal anti-human FH (The Binding Site, Birmingham, UK) or mouse anti-human FH monoclonal antibody (Serotec, Fiddlington, UK).

### Surface-Bound C3b-Binding Assay

The binding of FH to surface-bound C3b was determined using an ELISA method. Briefly, Nunc MaxiSorp ELISA plates were coated with  $62.5 \text{ ng}$  of purified C3b overnight at  $4^{\circ}\text{C}$ . After washing and blocking free reactive sites with PBS that contained 1% BSA, biotinylated purified FH at various concentrations was added for 1 h at room temperature alone or after preincubation with various concentrations of patient 1 or 2 purified IgG overnight at  $4^{\circ}\text{C}$ . After washing, the plates were incubated with a 1:1000 dilution of streptavidin biotinylated horseradish peroxidase for 30 min at room temperature. After additional washing, immobilized FH was revealed using the orthophenyldiamine substrate.

### Fluid-Phase C3b Co-factor Activity Assay

The fluid-phase co-factor activity of FH was determined in a C3b proteolytic assay using purified proteins. In brief,  $3 \mu\text{g}$  of biotinylated C3b was added to  $1 \mu\text{g}$  of factor I (Sigma Aldrich),  $1 \mu\text{g}$  of FH, and various amounts of purified IgG and was incubated at  $37^{\circ}\text{C}$  for 1 h in veronal buffered saline (pH 7.4). Proteolysis of C3b was evaluated by analyzing the cleavage of the  $\alpha$  chain of C3b and the generation of the  $\alpha 43/45\text{-kD}$  fragments using 8% SDS-PAGE under reducing conditions. Western blot analysis was performed on nitrocellulose membranes by using streptavidin biotinylated horseradish peroxidase and 4-chloro-1-naphthol and 1% hydrogen peroxide as substrates.

## Results

### Case Reports

Among the 48 children tested, three children exhibited a positive ELISA anti-FH assay. Clinical features are summarized in Table 1.

Table 1. Main clinical and biological features of the three children with anti-FH antibodies<sup>a</sup>

|   | Patient 1                  | Patient 2   | Patient 3   |
|---|----------------------------|---|---|
| Family history                            | None                       | None  | None  |
| Age at onset (yr)                         | 10                         | 3   | 9   |
| First symptoms                            |                            |   |   |
| fever                                     | +                          | –   | –   |
| vomiting                                  | +                          | –   | +   |
| diarrhea (bloody)                         | + (+)                      | +15 days before (–)   | –   |
| anuria/hypertension/edema                 | + / + / +                  | – / – / –   | – / + / –   |
| other                                     | Jaundice                   |   |   |
| Biological evaluation at the acute phase  |                            |   |   |
| hemoglobin (g/dl)                         | 5.1                        | 5.6   | 7.1   |
| schizocytes (%)                           | 13                         | 5   | 3   |
| platelet counts ( $\times 10^9/L$ )       | 51                         | 93  | 19  |
| creatinine ( $\mu\text{mol/L}$ )          | 1218                       | 113   | 236   |
| Evolution                                 |                            |   |   |
| recurrences                               | Yes: 2 in 1 mo             | Yes: 4 in 17 mo   | Yes: 3 in 3 mo  |
| extrarenal organs involvement             | Yes: liver, pancreas,      | Yes: heart, at the first No<br>relapse                                  | No  |
| end stage-renal failure                   | Yes: 2 mo after the onset  | No  | No  |
| hypertension                              | Yes: needing binephrectomy | Yes   | Yes   |
| Actual treatment                          | Chronic hemodialysis       | Plasma exchanges<br>1/3 wk<br>Antihypertensive<br>drugs<br>Azathioprine | Antihypertensive<br>drugs<br>Low dose of<br>glucocorticoids |
| Etiologic evaluation                      |                            |   |   |
| anti-LPS <i>Escherichia coli</i> antibody | Neg                        | Neg   | Neg   |
| stool Stx genes (PCR)                     | Neg                        | Neg   | Neg   |
| ADAMST-13 activity                        | Normal                     | Normal  | Normal  |
| plasma homocysteine                       | Normal                     | Normal  | Normal  |
| FH gene (23 exons)                        | Normal                     | Normal  | Normal  |
| Immunologic evaluation                    |                            |   |   |
| ANA titer, pattern                        | 1:320, speckled            | 1:100, speckled   | 1:200, speckled   |
| anti-DNA, anti-ENA                        | Neg, Neg                   | Neg, Neg  | Neg, Neg  |
| anti-cardiolipin, anti-B2gp1              | Neg, Neg                   | Neg, Neg  | Neg, Neg  |
| ANCA                                      | Neg                        | Neg   | Neg   |

<sup>a</sup>FH, factor H; Stx, shigatoxin; ANA, antinuclear antibodies; ANCA, antineutrophil cytoplasmic antibodies; ENA, extractable nuclear antigens.

**Patient 1.** A 10-yr-old girl was referred because of fever, headache, vomiting, and bloody diarrhea. On examination, the girl presented with jaundice, edema, oligo-anuria, and high BP. Routine laboratory tests showed pronounced hemolytic anemia (hemoglobin, 5.1 g/dl) with 13% schizocytes on peripheral blood smears, thrombocytopenia (platelets,  $51 \times 10^9/L$ ), and renal failure (serum creatinine, 1218  $\mu\text{mol/L}$ ). Urine output normalized after 24 d, and serum creatinine decreased to 260  $\mu\text{mol/L}$ . PCR of Stx in the stools and detection of serum antibodies against LPS (026, 091, 0103, 0111, 0128, 0145, and 0157) were negative. Biologic exploration revealed no causative factor (Table 1) but showed the presence of antinuclear antibodies (ANA; titers 1:320) with a speckled pattern without anti-extractable nuclear antigens (anti-ENA) antibody identified and no anti-double-stranded DNA (dsDNA) antibody. Immuno-

logic evaluation did not detect any other autoantibody. The evolution was marked by two recurrences of HUS over 1 mo, with involvement of extrarenal organs including cytolytic hepatitis, pancreatitis, pericarditis, and septic complications. The child was in ESRD after 2 mo despite a therapy associating antihypertensive treatment, transfusions, two fresh frozen plasma (FFP) infusions during the first relapse, and one intravenous IgG infusion (500 mg/kg) during the second relapse. Uncontrolled hypertension led to a bilateral nephrectomy 6 mo later. Histology of the nephrectomy specimen showed severe glomerular and arterial microangiopathy without deposits on immunohistochemistry staining.

**Patient 2.** A 3-yr-old boy was hospitalized because of anemia after an episode of diarrhea 15 d before. Biologic evaluation showed pronounced anemia (hemoglobin, 5.6 g/dl) with mild

thrombocytopenia (platelets,  $93 \times 10^9/L$ ) and renal involvement (serum creatinine,  $113 \mu\text{mol}/L$ ). PCR of Stx in the stools and detection of serum antibodies against LPS (seven serotypes tested including 0157:H7) were negative. He received one blood transfusion. Ten days later, he developed oliguric renal failure with hypertension and recurrence of anemia and thrombocytopenia that required blood transfusion, hemodialysis, and five plasma exchanges in 10 d. Renal biopsy showed mild glomerular and arterial microangiopathy. A recurrence 2 mo later was treated with seven plasma exchanges with FFP replacement in 21 d, which were progressively spaced out to one session every 3 wk during 4 mo. A second recurrence occurred 1 mo after, again requiring plasma exchanges during 6 mo. Four months after the last plasma exchange was a third recurrence, and plasma exchange treatment was introduced again associated with glucocorticoids at 1.4 mg/kg per d with progressive decrease. Biologic exploration revealed no causative factor (Table 1) but showed low titers (1:100) of ANA with a speckled pattern without ENA antibody and anti-dsDNA antibody. Immunologic evaluation did not detect any other autoantibody. Presently, 28 mo after the onset, the child remains on plasma exchange therapy once every 3 wk associated with 1.5 mg/kg per d azathioprine and antihypertensive treatment. Renal function remains normal.

**Patient 3.** A 9-yr-old boy was referred because of asthenia, pallor, vomiting, and abdominal pain without diarrhea. Biologic evaluation showed hemolytic anemia (hemoglobin, 7.1 g/dl, 3% schizocytes) with major thrombocytopenia (platelets,  $19 \times 10^9/L$ ) and renal failure (serum creatinine,  $236 \mu\text{mol}/L$ ). PCR of Stx in the stools and detection of serum antibodies against LPS (seven serotypes tested, including 0157:H7) were negative. He received blood transfusion. Twelve days later, he developed a relapse that was treated with plasma exchanges with FFP replacement, which were continued once a week during 9 mo because of two recurrences. A treatment with glucocorticoids was introduced at month 5 with very progressive decrease. Biologic exploration revealed no causative factor (Table 1) but showed low titers (1:200) of ANA with a speckled pattern without ENA antibody and anti-dsDNA antibody. Immunologic evaluation did not detect any other autoantibody, but antiplatelets antibodies were developed during the follow-up. Presently, 31 mo after the onset, he needs antihypertensive treatment with low doses of glucocorticoids and has normal renal function.

#### Complement Components Assessment

Plasma complement profiles of the three patients are depicted in Table 2. Patient 1 was investigated at the acute phase of the disease, and she exhibited complement consumption through the alternative pathway, as indicated by low plasma levels of the complement alternative pathway components C3 and factor B (FB). FH antigenic level was normal, whereas FH activity was profoundly decreased (10%). A few weeks later and during the 18 mo of follow-up, the child exhibited persistent low FH activity with normal FH antigenic level and decreased C3 and FB plasma levels. Complement components of patient 2 were investigated 2

Table 2. Results of complement investigation of the three patients at different selected times<sup>a</sup>

| Tests       | Patient 1 |        |         | Patient 2 |          |         | Patient 3 |          |         | Normal Ranges |          |                        |
|-------------|-----------|--------|---------|-----------|----------|---------|-----------|----------|---------|---------------|----------|------------------------|
|             | Day 0     | Day 15 | Month 7 | Month 14  | Month 17 | Month 2 | Month 15  | Month 27 | Month 3 |               | Month 24 | Month 29               |
| CH50        | 69        | 93     | 80      | 96        | 85       | 91      | 94        | 117      | 82      | 78            | 86       | 70-130%                |
| C3          | 460       | 638    | 610     | 647       | 630      | 978     | 785       | 771      | 594     | 574           | 630      | 660-1250 mg/L          |
| C4          | 130       | 163    | 116     | 88        | 112      | 129     | 188       | 156      | 176     | 127           | 124      | 93-380 mg/L            |
| Factor B    | 68        | 78     | 76      | 69        | 76       | 169     | 158       | 147      | 112     | 90            | 82       | 90-320 mg/L            |
| FH antigen  | 91        | ND     | ND      | 121       | 95       | 100     | 117       | 109      | 95      | 128           | 109      | 70-130%                |
| FH activity | 10        | ND     | 21      | 38        | 47       | ND      | ND        | 70       | ND      | 56            | 67       | 70-130%                |
| Anti-FH IgG | 2323      | 1812   | 1107    | 842       | 955      | 317     | 1593      | 1162     | 1830    | 1910          | 2160     | <1 AU <sup>b</sup> /ml |

<sup>a</sup>ND, not determined.

<sup>b</sup>Arbitrary units as defined by a reference positive plasma that was issue from a sample of patient 1 taken at month 6 of follow up.

mo after the onset of the disease and exhibited normal immunochemical levels that remained stable subsequently. Functional FH activity could not be investigated at the acute phase and was marginally decreased (70% of normal) when studied at 27 mo of follow-up. Patient 3 was investigated 3 mo after onset. Complement components exploration showed low C3 and normal antigenic FH levels at 3, 24, and 29 mo of follow-up. Functional FH activity was decreased at 56 and 67% of normal when studied at 24 and 29 mo, respectively. In the three patients, all exons of FH gene were analyzed by sequencing, and no abnormality was found.

*Anti-FH Antibody*

An antibody of IgG isotype reactive with FH was found in the plasma of the three patients by ELISA. The isotypes were subsequently identified as IgG3 in patient 1, IgG1 in patient 2, and IgG1 and 3 in patient 3 (Figure 1). The IgG fractions and the derived Fab'2 conserved the anti-FH specificity, whereas the plasma IgG depletion removed the anti-FH reactivity (Figure 2).

Plasma IgG binding on immobilized FH was found to be inhibited by incubation with purified FH and with sheep polyclonal anti-human FH, whereas the binding was not inhibited by a mouse monoclonal anti-FH antibody specific for a region that comprised SCR1 to 4, by neither nonspecific sheep polyclonal nor mouse monoclonal antibodies (Figure 3).

The 45 other children who presented with atypical HUS were negative for the test. We tested control samples, including samples from children who presented with typical HUS (*n* = 10) and patients who exhibited anti-DNA (*n* = 10) anti-cardiolipin (*n* = 10), antineutrophil cytoplasmic (*n* = 10), antiglomerular basement membrane (*n* = 10) or anti-alternative C3 convertase (*n* = 10) antibodies. None of them was found to be positive (Figure 4).

Anti-FH arbitrary units were defined using a reference positive sample. For patient 1, titers were high at the two first samples and decreased and then remained stable during the 19 mo of follow-up. After immunosuppressive treatment, anti-FH

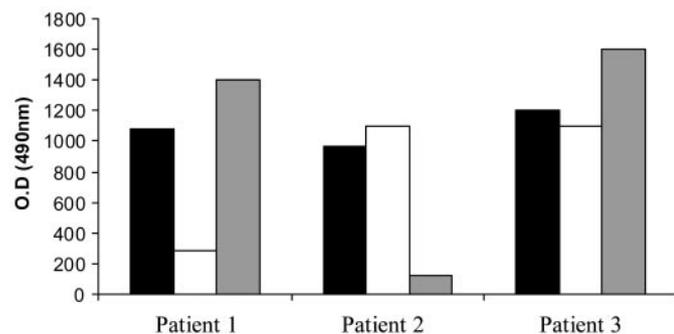
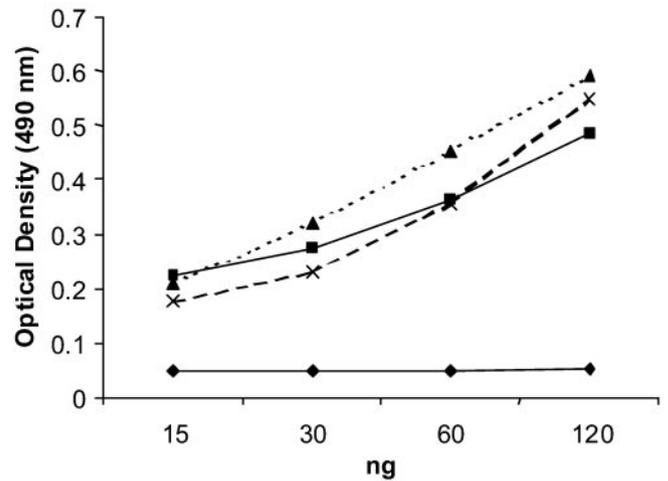
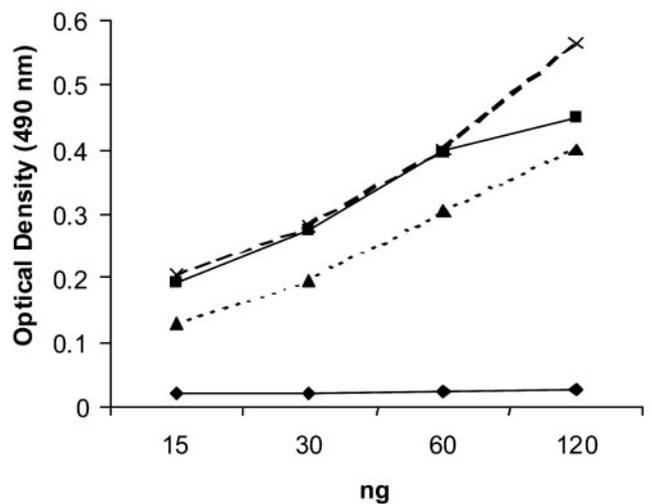


Figure 1. Isotype determination of the anti-factor H (anti-FH) antibodies of the three patients. The anti-FH binding was determined using 1:50 diluted plasma samples and anti-human IgG (■), IgG1 (□) and IgG3 (▨) antibodies. Anti-FH IgG were identified as IgG3 in patient 1, IgG1 in patient 2, and IgG1 and 3 in patient 3.



Patient 1



Patient 2

Figure 2. Anti-FH specificity of purified IgG and derived Fab'2 from patients' plasma. The plasma IgG (■), purified IgG (▲), and derived Fab'2 (×) exhibited the anti-FH specificity, whereas plasma IgG depletion (◆) removed the anti-FH reactivity.

antibody titers decreased, and concomitantly FH activity was found to rise to 80% of normal value. For patient 2 (Figure 5), titers were found to be higher at month 15 (2 mo after last plasma exchange and 2 mo before the third relapse) than at month 3 and mildly decreased at month 23 during plasma exchange therapy. At 30 mo of follow-up, plasma samples that were obtained before and after plasma exchange treatment were tested. The results showed a rise of functional FH activity from 68 to 140% of normal after plasma exchange. Concomitantly, anti-FH antibody titers were found to be significantly decreased when tested immediately after plasma exchange treatment at month 30 (Figure 5). Patient 3 exhibited stable titers of anti-FH IgG at months 3 and 24.

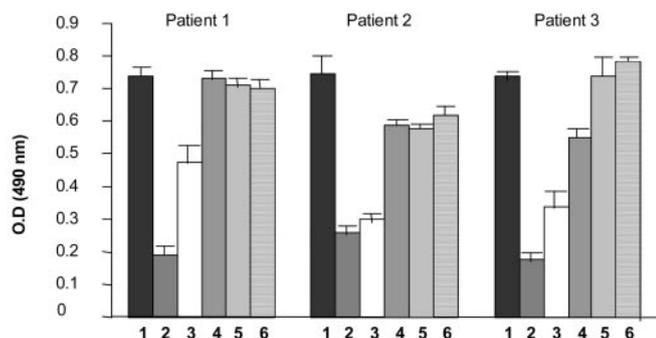


Figure 3. Inhibition of FH IgG binding by purified FH and sheep polyclonal anti-human FH antibody. Diluted plasma (1:500) of the three patients was submitted to the ELISA anti-FH assay, alone (lane 1) or with co-incubation with 1  $\mu$ g of purified FH (lane 2), polyclonal (lane 3) or monoclonal (lane 4) anti-FH antibodies, or nonspecific sheep polyclonal (lane 5) and nonspecific mouse monoclonal (lane 6) antibodies diluted at the same concentration.

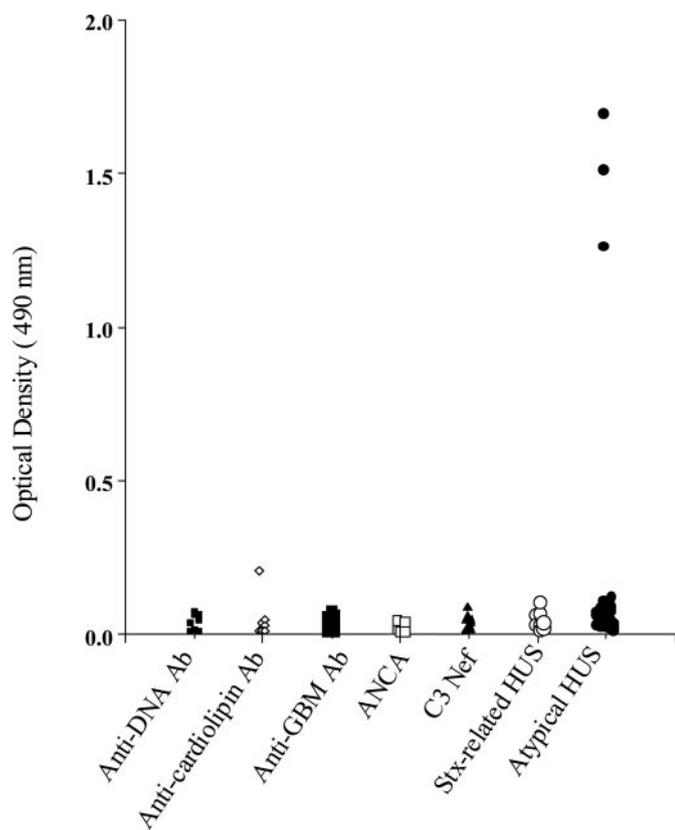


Figure 4. Results of the anti-FH ELISA assay in patients who presented with anti-DNA antibodies ( $n = 10$ ), anti-cardiolipin antibodies ( $n = 10$ ), anti-glomerular basement membrane antibodies ( $n = 10$ ), antineutrophil cytoplasmic antibodies (ANCA;  $n = 10$ ), anti-alternative C3 convertase (C3Nef;  $n = 10$ ) antibodies, and Stx-related hemolytic uremic syndrome (HUS;  $n = 10$ ) or atypical HUS ( $n = 48$ ).

#### *In Vitro* FH Functional Activity Assays

The binding of various concentrations of biotinylated purified FH to surface-bound C3b was tested in the presence of

patient 1 and 2 plasma-purified IgG. We observed a binding of increasing amounts of biotinylated FH to surface-bound C3b that was not disturbed by the IgG-containing anti-FH antibodies (Figure 6).

The co-factor activity of FH in the proteolysis of fluid-phase C3b by factor I was tested in presence of patient 1 and 2 plasma-purified IgG. This activity was similar to the activity of purified FH alone (data not shown).

## Discussion

We report for the first time on the presence of persistent anti-FH autoantibodies in a context of atypical HUS. Our results indicate that the anti-FH specificity is IgG dependent and is conserved by the Fab'2 fraction. Plasma IgG binding on FH was inhibited by a polyclonal anti-FH antibody but not by a mouse monoclonal anti-FH antibody specific for a region that comprised SCR1 to 4, showing evidence that the implicated epitopes are not localized in the N terminal domain of the FH protein. A monoclonal Ig  $\lambda$  light-chain dimer causing uncontrolled alternative pathway activation by binding to the SCR3 of FH was isolated previously from the serum of a patient with hypocomplementemic membranoproliferative glomerulonephritis (20). It is interesting that both atypical HUS and membranoproliferative glomerulonephritis have already been reported to be associated with homozygous FH deficiency (4,21).

No anti-FH antibody was found in a large series of plasma samples, including healthy control subjects or patients of various autoimmune disorders, suggesting that these antibodies are specific to particular forms of atypical HUS. These antibodies were found in three of 48 children who presented with non Stx-related HUS. Thus, the anti-FH antibody prevalence is approximately 6% in this study population as detected by our experimental conditions. The frequency of anti-FH antibody may be underestimated by the use of plastic-coated FH, which might hide some epitope of the protein and thus impair the detection of fluid-phase anti-FH antibody.

We chose to measure the FH activity by reference hemolytic test because this method allows the investigation of both the C3b binding capacity of FH and its activity of decay-dissociation of Bb from the preformed cell-bound nickel-stabilized C3 convertase (18). In our patients, plasma FH activity was found to be decreased, whereas plasma FH antigenic levels were normal and the FH gene analysis by exon-specific sequencing revealed no abnormality in all three patients. These results indicate that the presence of IgG anti-FH antibodies led to an acquired functional FH deficiency. As reported among hereditary FH-deficient individuals (4,22,23), two of three patients presented with low C3 levels, suggestive of mild alternative pathway-mediated complement activation.

The effect of the anti-FH antibodies on the capacity that the FH have to control activation of the complement system was estimated using three different assays: Analysis of the decay-accelerating activity of the alternative pathway C3 convertase, analysis of the binding of FH to surface-bound C3b, and analysis of the co-factor activity of FH in the proteolysis of fluid-phase C3b by factor I. The results showed perturbation of decay-accelerating activity in the three patients. This perturba-

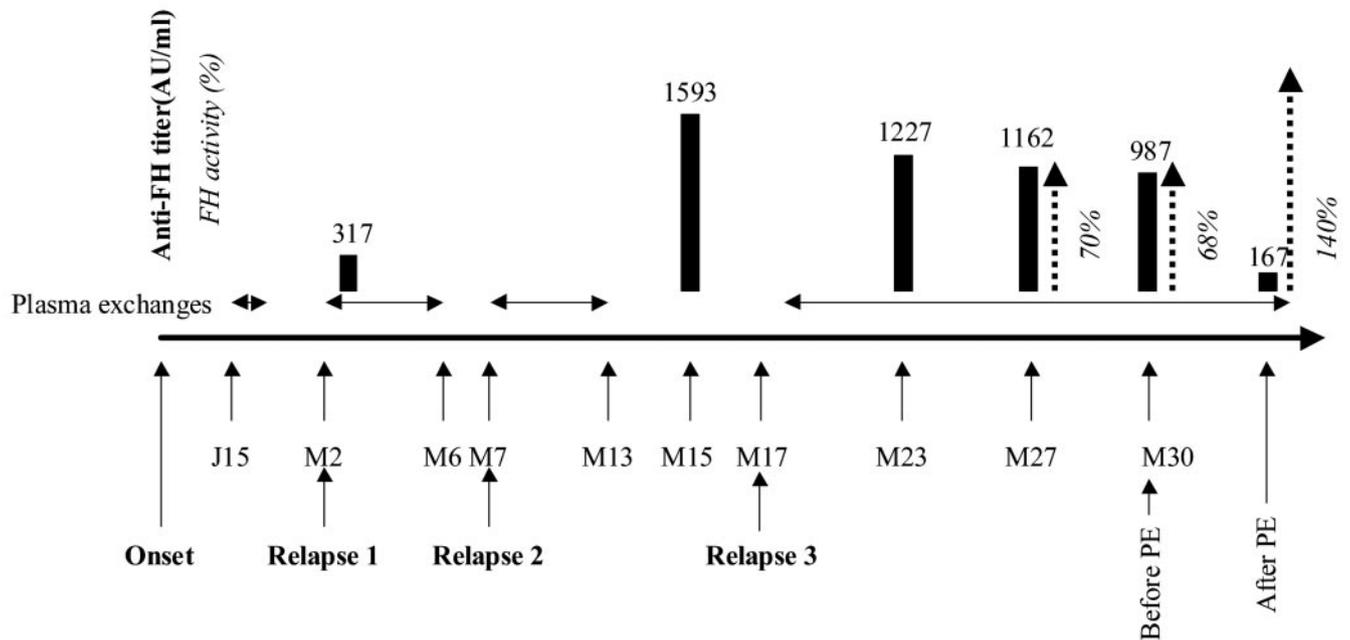


Figure 5. Evolution of the patient 2's anti-FH antibody titers during the progress of the disease. The plasma exchange therapy periods are indicated by double arrows. At month 30, samples were obtained immediately before and after plasma exchange, and FH function and anti-FH antibody titers were evaluated.

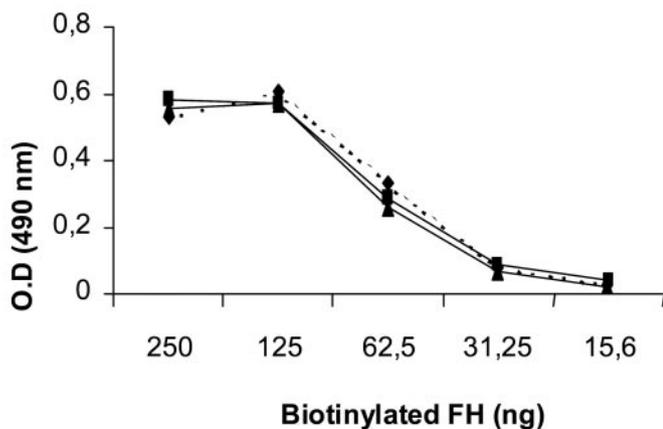


Figure 6. Biotinylated FH binding to surface-bound C3b in the presence of 500 ng of purified patient 1 (■), patient 2 (▲), or control nonspecific (◆) IgG.

tion was in correlation with the anti-FH antibody titers for patients 1 and 2. No inhibition by purified IgG-containing anti-FH antibodies of the FH capacity to bind surface-bound C3b was observed under our experimental conditions. As three distinct binding sites to C3b have been identified in FH, maybe a perturbation of one of the binding sites could not be detected by this assay. Co-factor activity of purified FH was not modified by an incubation with purified IgG-containing anti-FH antibodies. This result suggests that the epitopes recognized by the autoantibodies are not implicated in this function, which has been shown to be related to SCR1 to 4 (24). This is in agreement with our results demonstrating no inhibition of the binding of the anti-FH antibodies to immobilized FH by a

mouse monoclonal anti-FH antibody specific for a region that comprises SCR1 to 4.

We suggest that these autoantibodies probably influence the binding of FH to the C3b,Bb convertase, thereby compromising FH activity *in vivo*. This is not the first example of autoantibodies that do not neutralize the antigen activity. An example of nonneutralizing anti-ADAMST-13 antibodies was recently reported in a patient who had TTP and severely reduced ADAMST-13 activity but showed no ADAMST-13 inhibition in a fluid-phase activity assay (25). Epitope mapping of the anti-FH antibodies will be important for the understanding of the mechanisms of this FH inhibition *in vivo*.

Similar to the reported patients who carry an FH gene mutation (5-9), the clinical features of the three patients were recurrent HUS leading to end-stage renal failure at the second relapse in patient 1. A recurrent form of HUS is an essential criterion for an atypical form of HUS. Two of the three patients exhibited an episode of diarrhea. The presence of diarrhea is the main clinical criterion used for the classification between typical and atypical HUS, but it may be absent in a confirmed Stx-related HUS (26) and present in the other forms, as a result of microangiopathic lesions of the gut (21,23,27,28). For that reason, it may be preferable to use the term Stx-HUS rather than postdiarrheal HUS.

It is worth noting that all three patients exhibited ANA that may be related to this particular form of disease. A recent study reporting on 81 adult thrombotic microangiopathies revealed that ANA were highly frequent in idiopathic TMA (41.3%) and were frequently associated with an acquired ADAMST-13 deficiency (29). The association of ANA with organ-specific autoantibodies is frequently reported in various autoimmune dis-

orders, such as myasthenia gravis and autoimmune thyroiditis (30,31), pointing to the importance of immune dysregulation in the induction of these autoimmune diseases. None of the three patients had presented before and since the onset of HUS any symptom and any other biologic marker of systemic autoimmune disease such as systemic lupus erythematosus.

Although beneficial effect of plasma exchanges is unproved in atypical HUS in contrast to TTP (1,32), this therapy has been effective in two patients, maybe because of the removal of the anti-FH antibodies. Both patients remain without recurrence with an immunosuppressive treatment using azathioprine in one case and glucocorticoids in the other. The identification of anti-FH antibodies may lead to the design of new approaches of diagnosis and treatment with a particular interest in plasma exchanges or immunosuppressive therapies.

Similar to the observations of acquired von Willebrand factor-cleaving protease deficiencies secondary to IgG antibodies in patients who exhibit TTP, our report provides evidence for the first time that HUS may occur in a context of an autoimmune disease with the development of anti-FH-specific antibodies leading to an acquired FH deficiency. This new mechanism of functional FH deficiency emphasizes the role of the alternative pathway regulatory function of FH in the process of the illness.

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