Functional analyses indicate a pathogenic role of factor H autoantibodies in atypical haemolytic uraemic syndrome

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Abstract

Background. Atypical haemolytic uraemic syndrome (aHUS) is associated with defective complement regulation. Recently, an autoimmune aHUS form has been described that is associated with complement factor H (CFH) autoantibodies. The aim of this study was to address the pathologic relevance of CFH autoantibodies in aHUS.

Methods. CFH autoantibodies were identified and antibody levels were analysed in three aHUS patients during the disease course by the ELISA method. Epitope mapping was performed using recombinant factor H fragments and domain-mapped monoclonal antibodies. The effect of the antibodies on cell-protective activity of CFH was measured by haemolytic assays. CFH:antibody complexes were analysed by ELISA.

Results. All three autoantibodies bound to the C-terminal domain of CFH, which is essential for CFH binding to cell surfaces. In patient 1, plasma exchanges and immune adsorption temporarily reduced the autoantibody titre and led to temporary clinical improvement. In patient 2, plasma exchanges and long-term immunosuppression strongly reduced the CFH autoantibody level, and induced a stable remission of aHUS. Patient 3 had lower autoantibody levels that decreased during the follow-up and is in good clinical condition. The patients' plasma samples caused enhanced lysis of sheep erythrocytes, and the degree of lysis correlated with the CFH autoantibody titre and the amount of CFH:antibody complexes. An addition of purified CFH to aHUS plasma or removal of IgG inhibited the haemolytic activity.

Conclusion. These results support a direct role of the autoantibodies in aHUS pathology by inhibiting the regulatory function of CFH at cell surfaces and suggest that reduction of the autoantibody titre is beneficial for the patients.

Keywords: autoantibody; complement; factor H; haemolytic uraemic syndrome

Introduction

Haemolytic uraemic syndrome (HUS) is a severe kidney disease characterized by microangiopathic haemolytic anaemia, low platelet count and acute renal failure. The
common form of this disease (D+ HUS) is usually caused by bacterial infection and is associated with diarrhoea [1]. The more rare, atypical form of HUS (aHUS or D− HUS) includes both familial and sporadic cases, and is associated with dysregulation of complement activation [1,2]. In aHUS patients, disease-associated mutations and polymorphisms in complement genes have been described for factor H (CFH), membrane cofactor protein (MCP), factor I (CHI) and factors B and C3 [3,4]. The mutations are generally heterozygous, and most likely, the mutant proteins cause inappropriate local control of complement activation. Deletion of the factor H-related genes CFHR1 and CFHR3 is also associated with aHUS [5]. In addition, CFH autoantibody-associated aHUS, which has been reported in 6−10% of aHUS patients, represents an acquired autoimmune form of the disease [6,7]. The appearance of CFH-specific autoantibodies is linked with the deletion of CFHR1 and CFHR3 [8].

CFH is an abundant plasma protein and the major regulator of the alternative complement pathway [9]. CFH is composed of 20 short consensus repeat (SCR) domains. The N-terminal domains (SCRs 1−4) of CFH mediate the cofactor and decay accelerating activities [10,11], and the C-terminal domains (SCRs 19−20) contain binding sites for C3b, heparin and endothelial cells [12−14]. Thus, the complement regulatory region and the recognition region responsible for the cell attachment of CFH are spatially separated. SCRs 19−20 represent a hot spot for aHUS-associated CFH mutations [15]. Several of these mutations have been shown to reduce CFH binding to C3b and to cellular surfaces [16−18]. Functional studies have demonstrated that an intact C-terminus is necessary for CFH to bind and exert its complement regulatory activity on cell and tissue surfaces, thus, for full-scale protection of host cells against the activated complement system [19−22].

We have previously mapped the binding sites of CFH autoantibodies to the same C-terminal domains of CFH, i.e. SCRs 19−20, where most of the aHUS-associated CFH mutations occur. In an in vitro haemolytic assay, the autoantibody-positive plasma of one patient caused enhanced lysis of sheep erythrocytes [7]. This suggests that CFH autoantibodies inhibit CFH complement regulatory activity on cell surfaces.

In order to elaborate on the suggested pathomechanism and to assess the clinical significance of CFH autoantibodies in aHUS, we have followed the titre and activity of the autoantibodies during the disease course of aHUS patients.

Subjects and methods
The studies have been approved by the Research Ethics Committee of the Medical Faculty of Friedrich Schiller University and were performed in accordance with the Declaration of Helsinki.

Sample collection and analyses of anti-CFH autoantibodies
Plasma samples, collected with informed consent, were analysed for the presence of CFHR1 and CFHR3 by Western blot [8]. CFH autoantibodies were identified as described [7]. The IgG isotypes were determined using IgG1-, IgG2-, IgG3- and IgG4-specific mouse mAbs (Sigma-Aldrich, Taufkirchen, Germany) and HRP-conjugated rabbit anti-mouse Ig (DakoCytomation, Hamburg, Germany). IgG was isolated from plasma using Protein G columns (GE Healthcare, Freiburg, Germany). The binding domains of the autoantibodies were determined using recombinant CFH fragments as described [7]. For inhibition experiments, immobilized CFH was preincubated with CFH-specific goat antisera (Merek Biosciences, Schwabach, Germany), rabbit antisera raised against SCRs 1−4 [23] and SCRs 19−20 of CFH [24] (both diluted 1:100) or with domain mapped, CFH-specific monoclonal antibodies [7,14].

Determination of CFH autoantibody titre in patient plasma
The plasma samples were incubated on CFH-coated wells in serial dilutions. The titre was determined in comparison with a reference sample of patient 1, taken 10 months after initial disease onset, and is expressed in arbitrary units as described by Dragon-Durey et al. [6]. This sample was compared to a reference with 1000 AU/ml [6], kindly provided by Dr Dragon-Durey, and had essentially the same titre (1014 AU/ml).

Haemolysis assays
Haemolysis assays were performed as described using sheep erythrocytes (BioTrend Chemikalien, Cologne, Germany) [7]. Some samples were incubated with Protein G beads for 5−15 min at 20 °C to deplete IgG. Haemolytic effects of the CFH-specific mAbs (50 µg/ml) were measured using 10% C2-depleted plasma (Merek) in order to prevent the activation of the classical complement pathway.

Measurement of autoantibody:CFH complexes
Immune complexes were captured on microtitre plate wells coated with the anti-CFH mAb M15 at 5 µg/ml. After blocking, serial plasma dilutions were incubated for 1 h at 20 °C and bound complexes were detected using HRP-conjugated anti-human IgG (Sigma-Aldrich). Bound CFH was detected using a CFH antisera.

Results
Case descriptions
Patient 1. Patient 1, a 10-year-old boy (patient #564 in ref. 7), was admitted to hospital at the age of 6.5 years with kidney failure and was later diagnosed with aHUS. ADAMTS13 activity was normal. Both serum and stool tests were negative for enterohaemorrhagic E. coli. Two weeks after disease onset, he received 10 plasmaphereses in 19 days. Disease recurrence with the increase of creatinine, low complement C3 levels and increased C3d occurred 19 days. A second recurrence occurred 3 months later. Plasma infusions (three times in 10-day intervals) did not improve renal function; therefore, the presence of CFH autoantibodies was postulated. Plasma exchange was resumed (10 times in 13 days) with haemodialysis three times. Additionally, immunosuppressive therapy was initiated (mycophenolate mofetil 2 × 250 mg/day, later 2 × 500 mg/day for 2 months). Rituximab (375 mg/m²) was applied three times at 3-week intervals started at 11 months. Because of unsatisfactory efficacy, this was followed by cyclophosphamide treatment three times at 3-week intervals. Plasma exchanges were performed six times within 1 week at 12 months, then seven times in 12 days at 14 months. A short trial with immunoadsorption was stopped because of an allergic reaction. Forty-two months after disease onset, the patient remained on peritoneal dialysis, which was changed...
from haemodialysis because of catheter infection and sepsis. It is of note that C3 and C3d levels were normal. Genetic analysis showed that in the CFH gene, this patient was homozygous for the C allele of the c.921A > G polymorphism (A307A in SCR5) [25,26] and had the H4 haplotype [27]. In the CFH gene, a heterozygous G818A transition (S268R) was found, which is a non-disease-causing polymorphism [28]. The patient lacked CFHR1 and CFHR3.

**Patient 2.** Patient 2 is an 8.5-year-old girl, who presented with D-HUS at the age of 6.8 years. She was treated with seven plasma exchanges and received immunosuppressive therapy with mycophenolate mofetil and prednisolone. Remission of aHUS was achieved and renal function returned to normal. On immunosuppression and antihypertensive treatment, she has remained free from recurrences up to now. The patient lacks the CFHR1 and CFHR3 proteins.

**Patient 3.** Patient 3 is a 12.5-year-old boy who presented with D-HUS at the age of 5 years. At the early phase of the disease, he was treated with haemodialysis and received plasmapheresis on every second day (seven sessions) followed by weekly transfusion of fresh frozen plasma for a period of 3 months. Her kidney function rapidly improved and has since been stable with endogenous creatinine clearance of 35–50 ml/min/1.73 m². He is in good clinical condition with normal complement parameters normalized. Patient 3 had a lower autoantibody level that decreased during the follow-up (Figure 2C) and remained in good clinical condition with normal complement levels.

**Characterization of the CFH autoantibodies**

All three patients, but not their family members, have CFH autoantibodies (Figure 1, data not shown). The anti-CFH IgG of patient 1 was characterized before [7]. The three autoantibodies were of IgG3 isotype. Reactivity of the autoantibodies with recombinant CFH fragments was measured by ELISA in order to localize the binding domain. The autoantibodies bound only to the CFH fragment that includes the most C-terminal SCRs20 domain, i.e. SCjHjs 15–20. Binding of the autoantibodies to CFH was inhibited by C-terminal-binding mAbs (C18, C14 and C02), but not by mAbs that recognize the N-terminus (N11) or the middle region of CFH (M12, M13, M15). Likewise, polyclonal antibodies against SCRs 19–20 and against full-length CFH reduced autoantibody:CFH binding, whereas an antibody specific to SCRs 1–4 did not affect autoantibody binding to CFH (Figure 1, data not shown). No complete inhibition of autoantibody binding could be achieved with mAb C02 in the case of patient 2, indicating that despite the same binding domain on CFH, the exact binding epitope is different in this patient. Thus, CFH autoantibodies from the three unrelated patients have similar characteristics, because they are of the same isotype and bind to the same domain on CFH.

**CFH autoantibody levels during disease course**

To assess the relevance of the CFH autoantibodies in aHUS, the autoantibody titre was followed during treatment of the patients. In patient 1, plasma exchanges temporarily reduced the CFH autoantibody titre (Figure 2A), which was accompanied by temporarily normalized C3 levels and improvement in clinical parameters (Table 1). Immunosuppressive therapy was attempted to reduce the amount of circulating anti-CFH IgG, but the titre remained high. Although immune adsorption was effective in reducing the CFH autoantibody titre (from 982 to 219 AU/ml), the treatment had to be stopped because of allergic reaction. This was followed by an increase in circulating autoantibodies and the patient’s kidney function deteriorated. At present, the patient has persistently high titres (707–1244 AU/ml in the last 6 months) and remains on peritoneal dialysis. In patient 2, the autoantibody levels were strongly reduced after initial plasma exchanges and immunosuppressive treatment and remained low with little variation during the follow-up period (<100 AU/ml in the last 15 months) (Figure 2B). C3 level and clinical parameters normalized. Patient 3 had a lower autoantibody level that decreased during the follow-up (Figure 2C) and remained in good clinical condition with normal complement levels.

**CFH autoantibodies inhibit CFH cell-protective activity**

To elaborate on the suspected inhibitory role of the autoantibodies in protection of cellular surfaces by CFH against complement attack, haemolysis of sheep red blood cells (SRBC) was measured in the presence in patient plasma as described [7]. This assay allows measurement of complement alternative pathway activity on cell surfaces. Incubation with CFH autoantibody-positive plasma samples resulted in enhanced haemolysis of SRBC in all tested cases (n = 6), including the three patients and additional samples from patients described before (Figure 3 A) [7,8]. The sample of patient 3 with relatively low autoantibody titre showed the least haemolytic effect. In contrast, normal human plasma samples showed the background lytic effect. SRBC lysis was dose-dependently inhibited by the addition of purified CFH in patient plasma in all cases (data not shown). To determine if CFHR1/CFHR3 deficiency alone influences haemolytic activity, additional assays were performed with samples of CFHR1/CFHR3-deficient individuals who lack autoantibodies. These samples caused no enhanced haemolysis (Figure 3B). The role of CFH was further confirmed using CFH-specific mAbs. Among these antibodies, only the C-terminal-binding mAbs C18, C02 and C14, which have overlapping binding epitopes on CFH and compete with the autoantibodies for CFH binding (Figure 1) [7], caused enhanced SRBC lysis when added to normal human plasma (data not shown) or to C2-depleted plasma, which was used to exclude classical pathway-mediated lysis (Figure 3C).
Role of factor H autoantibodies in HUS

Fig. 1. Characterization of CFH autoantibodies of patients 2 and 3. (A) CFH autoantibodies were determined by ELISA. The binding of IgG from plasma samples to CFH and to BSA as a control was compared to exclude false positivity, as in the case of the sister of patient 3. Unrelated control samples (NHS1 and NHS2) were used as negative controls. (B) Determination of IgG isotype of the autoantibodies using CFH-coated plates and isotype-specific mAbs. As positive control, binding of the isotype-specific mAbs on immobilized human IgG is shown. (C) Binding of the autoantibodies to immobilized recombinant CFH fragments representing various SCR domains and covering the full length of CFH and to purified CFH. (D) Competition assay with CFH-specific antibodies raised against the whole molecule (anti-CFH), against SCRs 1–4 (anti-1–4) or SCRs 19–20 (anti-19–20), or with monoclonal antibodies binding to the C-terminus (C18, C14, C02), the middle region (M15) or N-terminus (N11) of CFH. Data of a representative experiment are shown.

The in vitro haemolytic activity of aHUS plasma correlates with the autoantibody titre

We next addressed the question whether the different autoantibody titres indeed result in variation of haemolytic activity in patient plasma. Samples taken at different time points from aHUS patients were analysed for both CFH autoantibody titre and haemolytic activity. The extent of SRBC lysis in all three analysed cases followed the changes in autoantibody titre and was lower when the titre dropped (Figure 4). The isotype and the binding epitope of the autoantibodies were the same at the analysed time points (data not shown). Because large differences in the C3 and CFH levels in the samples may influence the extent of haemolysis [19], these parameters were also determined. The C3 and CFH values of the analysed samples showed no correlation with haemolysis (Figure 4).

To directly address the role of the autoantibodies, the lytic effect in patient plasma before and after IgG depletion was compared. Preincubation in patient plasma with protein G beads resulted in reduced SRBC lysis, depending on the duration and thus the extent of IgG depletion, and reached background level after 15-min depletion (Figure 5A). This IgG depletion results in an artificial reduction of autoantibody titres, and the data indicate that the same plasma samples with the same C3 and CFH levels cause haemolysis to different extents depending on the amounts of autoantibodies remaining in the plasma. The same results were obtained with all three analysed autoantibody-positive in patient plasma samples. Furthermore, when adding the C-terminal-binding C18 mAb to the IgG-depleted plasma or to control normal human plasma, the lysis increased again (Figure 5B). This also shows that after IgG depletion, the complement system in the plasma remains active, and the reduced lysis is due to the removal of anti-CFH IgG and not due to an unspecific complement inhibitory effect. Thus, inhibition of CFH surface recognition functions of the autoantibodies results in inappropriate protection of cells against complement attack.
Fig. 2. Serum autoantibody titres in the patients during disease progression. (A) Patient 1 was diagnosed with aHUS and later experienced disease recurrences, during which he received plasma exchanges (horizontal bars). CFH autoantibody titres (in arbitrary units/ml) are shown for several available time points as vertical bars. As reference, a plasma sample taken at Month 10 was used (1000 AU/ml). Additional information about the treatment is indicated above by arrows. (B) and (C) show the titres during disease follow-up in patients 2 and 3, respectively, in relation to the reference plasma of patient 1. *The CFH autoantibody titre of these samples was determined retrospectively. PEx: plasma exchange, FFP: fresh frozen plasma.

Table 1. Laboratory parameters of patient 1 at selected time points

<table>
<thead>
<tr>
<th>Time point after disease onset</th>
<th>Anti-CFH IgG (AU/ml) (&lt;100)*</th>
<th>C3 (mg/ml) (0.7–1.2)*</th>
<th>C3d (mU/l) (&lt;40)*</th>
<th>Platelet count (×10^9/l) (150–450)*</th>
<th>LDH (IU/l) (105–333)*</th>
<th>Serum creatinine (mg/dl) (0.2–1.0)*</th>
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<tr>
<td>At onset</td>
<td>2282</td>
<td>0.8</td>
<td>60</td>
<td>36</td>
<td>2336</td>
<td>2.16</td>
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<tr>
<td>Month 9.5</td>
<td>ND</td>
<td>0.6</td>
<td>ND</td>
<td>41</td>
<td>240</td>
<td>3.46</td>
</tr>
<tr>
<td>Month 10, after PEx</td>
<td>1132</td>
<td>0.6</td>
<td>62</td>
<td>331</td>
<td>240</td>
<td>2.80</td>
</tr>
<tr>
<td>Month 12</td>
<td>2116</td>
<td>0.7</td>
<td>41</td>
<td>243</td>
<td>229</td>
<td>2.56</td>
</tr>
<tr>
<td>Month 12, after PEx</td>
<td>806</td>
<td>0.7</td>
<td>26</td>
<td>329</td>
<td>209</td>
<td>2.69</td>
</tr>
<tr>
<td>Month 14</td>
<td>1487</td>
<td>0.3</td>
<td>ND</td>
<td>146</td>
<td>425</td>
<td>3.70</td>
</tr>
<tr>
<td>Month 14, after PEx</td>
<td>675</td>
<td>0.8</td>
<td>29</td>
<td>292</td>
<td>307</td>
<td>2.90</td>
</tr>
</tbody>
</table>

ND, not determined; PEx, plasma exchange therapy.
Anti-CFH IgG titre is expressed in arbitrary units (AU/ml).
*Normal ranges.

CFH:autoantibody complexes in patient plasma

Analysis of autoantibody titres as mentioned above determines only free IgG autoantibodies that recognize immobilized CFH. To analyse CFH:anti-CFH complexes in patient plasma, serial dilutions of plasma samples were incubated in microtitre plate wells coated with the anti-CFH mAb M15 that binds outside the CFH C-terminus (within SCRs 11–18) [14], and immune complexes were detected with anti-human IgG. A dose-dependent binding of the CFH:autoantibody complexes was observed, and at
Fig. 3. In vitro haemolytic activity of autoantibody-positive plasma samples using the sheep erythrocyte lysis assay. (A) Sheep erythrocytes suffer dose-dependent lysis in the presence of autoantibody-positive patient plasma as compared to normal human plasma. Autoantibody titres are indicated in brackets; control samples had values <100 AU/ml. Haemoglobin release was measured in the supernatant by absorbance at 414 nm after 30 min incubation at 37 °C. Data represent mean values of duplicate measurements from a representative experiment and are expressed as percentage of total lysis achieved by H2O. (B) Plasma samples lacking CFHR1 and CFHR3 cause no haemolysis if there are no CFH autoantibodies present. CFHR1+/CFHR3+ normal plasma and plasma of the autoantibody positive, CFHR1−/CFHR3− patient 1 are shown as negative and positive controls, respectively. The addition of 20 µg/ml C18 mAb results in enhanced lysis in all samples (filled bars). (C) C-terminal-binding mAbs, which have overlapping epitopes and compete with the autoantibodies for CFH binding, cause enhanced haemolysis of sheep erythrocytes (mAbs C02, C14 and C18) incubated with 10% C2-depleted human plasma (used to exclude classical pathway-mediated lysis). In contrast, CFH-specific mAbs that bind outside the C-terminal domains (SCRs 19–20) cause no haemolysis (mAbs N11, N22, M12, M13 and M16).

Discussion

Experimental evidence and genetic analyses over the past years established that the delicate balance of complement activation and regulation is disturbed in the affected individuals and is involved in the pathogenesis of aHUS [1–3]. Apparently, aHUS is a multifactorial disease, with multiple predisposing genetic factors and the requirement of an environmental trigger, most often infection [29].

The role of the C-terminus in targeting CFH complement regulatory activity to host cells is supported by functional studies [20–22] and by a mouse model of aHUS [30]. In addition, sera derived from aHUS patients with C-terminal CFH mutations show reduced protection of sheep erythrocytes in a complement-dependent lysis assay [19,31].

The CFH autoantibodies analysed in this and in earlier studies bind to the most C-terminal domain of CFH, i.e. SCR20 (Figure 1) [7,8]. Cell survival assays show that aHUS-associated autoantibodies cause similar functional defects to those described for mutant CFH proteins and affect C-terminus-dependent complement regulatory function of CFH. SRBCs, which have surface polyanionic molecules similar to human cells and which lack human membrane complement regulators rely on CFH binding and CFH regulatory activity to evade human complement. Thus, when using human plasma in haemolytic assays, sheep erythrocytes are protected by bound human CFH and are not lysed. By contrast, plasma samples of aHUS patients that contain blocking autoantibodies cause haemolysis (Figure 3A). This in vitro haemolytic effect can be inhibited by the addition of excess CFH, or by depletion of IgG from the plasma that indicates normalized CFH.
Fig. 4. The in vitro haemolytic activity of patients’ plasma correlate with the autoantibody titre. (A) Patient 1 displayed persisting high autoantibody titres and also high haemolytic activity. These complement active samples were only available when the patient was already on peritoneal dialysis. Both C3 levels (0.8–1.3 mg/ml) and CFH levels (102–133%) were in the normal range in these samples. (B) Haemolytic activity of plasma samples of patient 2 taken at various time points. After the acute phase, both autoantibody titres and haemolytic activities are low. Sample 1 had a lower C3 level (0.42 mg/ml), whereas C3 levels were normal in the other samples (1.19–1.51 mg/ml). CFH levels were in the normal range (105–123%), except for sample 2 (61%). (C) Comparison of haemolytic activity of two plasma samples of a patient, described before [8], with high and low CFH autoantibody titres. C3 levels were 0.85 mg/ml and 1.3 mg/ml, and CFH levels 95% and 155% in samples 1 and 2, respectively. Plasma samples were used at 20% dilution in (A) and (B) and at 30% in (C).

The regulatory function on cellular surfaces after removal of CFH autoantibodies (Figure 5).

The same haemolytic effects are achieved through mAbs that share binding epitopes with the autoantibodies (Figure 3C). Reduced C3b binding of CFH and impaired C3b inactivation on the cell surface, which results in enhanced complement activation and cell damage, are caused by mAbs C02, C18 and C14 [21]. These mAbs cause enhanced SRBC lysis when added to normal human plasma or to IgG-depleted plasma of autoantibody-positive aHUS patients (Figure 5). Altogether, these data point to the importance of cell protection by surface-bound CFH under normal, physiological conditions and show that this mechanism does not function properly in aHUS patients with C-terminal CFH mutations or with CFH autoantibodies. The fenestrated endothelium in the kidney appears particularly sensitive to self-attack, thus the loss of full-scale complement control due to CFH dysfunction leads to complement-mediated damage [32].

The haemolytic assay is a useful tool to analyse CFH functional defects, either caused by C-terminal mutations or by autoantibodies. This assay has the advantage that it directly evaluates the cell surface activity of CFH, which is relevant in aHUS [7,19,31]. In contrast, fluid-phase assays may not be informative, because CFH function in plasma is usually normal in the patients [6,7,22]. However, the haemolytic assay can lead to false negative results, for example, if C3 is largely consumed, and therefore, the plasma has low complement activity. Thus, the extent of lysis induced by plasma samples depends on the autoantibody titre and affinity, the individual CFH level and the complement status, and it is important to collect and handle the samples properly so that they are suitable for such assays. In the analysed samples, CFH levels varied only little, and C3 levels were sufficient for complement activation, as shown by the use of mAb C18, and the haemolytic effect was dependent on the presence and amounts of autoantibodies in plasma (Figures 3–5).

The three autoantibodies analysed here had the same characteristics, because they were of the same isotype and bound on the same domain of CFH. There were differences, however, between the patients regarding the time of diagnosis of the autoantibodies and the applied treatment. In the two patients, where the autoantibody levels were permanently reduced, there was also improvement in kidney function. In patient 1, autoantibodies persist at high levels despite several attempted treatments and the patient progressed to end-stage renal failure.

The direct role of the autoantibodies in inhibiting CFH cell-protective effect and correlation of the autoantibody titre with cell-damaging effects (Figures 3–5) suggest that attempts to remove the autoantibodies from the patients’ circulation represent a rational therapeutic approach. Recently, plasma exchanges and rituximab were successfully applied before kidney transplantation of an aHUS patient with CFH autoantibodies [33]. Similarly, in a patient plasma exchanges and immunosuppression as post-transplant management resulted in the disappearance of autoantibodies and a favourable outcome [34]. In addition, purified or plasma-derived CFH might be beneficial by increasing the concentration of antibody-free CFH. Plasma exchanges, although they temporarily remove CFH autoantibodies (Figure 2 and
Role of factor H autoantibodies in HUS

Fig. 5. IgG depletion provides direct evidence for the CFH function-inhibitory effect of the autoantibodies. (A) Depletion of IgG by incubating plasma samples with Protein G for various time periods results in artificial titre reduction and reduced haemolytic activity. A representative experiment with samples of patient 2 is shown. (B) All three analysed in patient plasma samples showed haemolytic activity reduced to the control level after IgG depletion. Addition of CFH blocking mAb C18 to the depleted plasma reverts the beneficial effect of IgG depletion (plasma \( \Delta \operatorname{IgG} + \operatorname{C18} \)), indicating that IgG depletion removes CFH inhibitory antibodies, but the plasma samples still have a functional complement system.

Table 1) [6,33,34], might also have a boosting effect in the long term on autoantibody production by introducing a free CFH antigen. Aiming to reduce the amount of autoantibodies, by e.g. immunosuppressive therapies and/or immune adsorption, and intravenous IgG (IVIg) infusion, likely represents a better option and eliminates the above risk. It remains to be shown how and when CFH autoantibodies appear, and whether autoantibody generation is directly influenced by the lack of the CFHR1 and CFHR3 proteins.

Even though we could analyse only a few cases in detail in the present study, the results support a pathogenic role of the CFH autoantibodies in aHUS. Because of the similar binding sites and haemolytic effects of the autoantibodies analysed so far [7,8,31], these results likely apply in general. Further clinical studies involving more patients are required to better understand the role of autoantibodies and to determine which treatment is most suitable and effective in this patient group.

In conclusion, the data reported here demonstrate that there is a direct connection between the presence and the level of CFH autoantibodies and the extent of CFH functional defect at cell surfaces, indicating that CFH autoantibodies contribute to the pathology of aHUS.

Fig. 6. Analysis of CFH: autoantibody complexes in patient plasma. (A) The indicated dilutions of plasma samples, patient 2/I (autoantibody titre 1325 AU/ml), patient 2/II (361 AU/ml), patient 3/I (883 AU/ml) and 3/II (867 AU/ml), were incubated in wells coated with the anti-CFH mAb M15 as a catch antibody, and the bound CFH: autoantibody complexes were detected with anti-human IgG. As a control, normal human plasma (NHP) is also shown. (B) Immune complexes (left panel) are absent from IgG-depleted plasma (empty bars, \( \Delta \operatorname{IgG} \)), measured as in (A). As controls, binding of anti-IgG to plasma samples incubated in wells without anti-CFH mAb coat and the presence of CFH in the plasma samples (right panel) are shown. (C) The mAb C18 captures free CFH only, due to overlapping binding sites with the autoantibodies, thus falsely indicates low CFH levels if the autoantibody titre is high. In contrast, mAb M15 detects free CFH as well as CFH:IgG complexes, thus comparison of CFH levels determined using the two mAbs allows an estimate of free and complexed CFH.

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Conflict of interest statement. None declared.

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