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The C-terminus of complement factor H is essential for host cell protection[☆]

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Abstract

Complement is a powerful self-amplifying system of innate immune defense with the capacity to eliminate microbes directly. Factor H is a central regulator in plasma which protects host tissue from complement mediated damage. Here we characterize the relevance of surface attached factor H, and study the regulatory activity of factor H on endothelial cells. Although these cells expressed membrane bound regulators, cell bound factor H contributed substantially to complement regulatory activity at the cell surface. Blockade of the C-terminus of factor H with monoclonal antibodies inhibited cell binding of this soluble regulator and resulted in enhanced complement activation on the cells. In the absence of factor H, increased deposition and slower inactivation of C3b resulted in higher amount of membrane attack complexes on the cell surface. When the membrane regulators CD55 and CD59 were removed by enzymatic treatment, complement mediated cell lysis was enhanced in the absence of factor H. Importantly, inhibition of the C-terminus did not compromise the regulatory function of factor H in fluid phase. Altogether these data point to a highly relevant, yet so far underestimated role of factor H for complement control at cellular surfaces, and reveal a decisive role of the factor H C-terminus in host cell recognition and protection.
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1. Introduction

Complement is an essential defense system of innate immunity. On foreign surfaces, such as microbes, complement activation is favoured to initiate elimination of these non-self particles. At the same time, host cells must be protected from complement attack to minimize damage to host tissue. To this end, the human body utilizes both fluid phase and membrane bound regulators to limit complement activation both in time and space (Walport, 2001).

The alternative pathway of complement is continuously activated via the so-called tick-over mechanism and the activation product C3b binds to surfaces in an indiscriminatory manner. If left uncontrolled, surface-deposited C3b allows generation of more C3b (amplification step), and initiates effector functions including opsonization and activation of the late complement components, which results in the assembly of the terminal membrane attack complex (MAC) and in cell lysis. Self-cells express integral membrane proteins in different combination and number that control complement activation. These membrane bound regulators include CD35/CR1 (complement receptor type 1), CD46/MCP (membrane cofactor protein) and CD55/DAF (decay accelerating factor), which all promote C3b inactivation. CD59 acts at a later phase and prevents MAC formation. In addition, host cells display polyanionic molecules which allow discrimination of self from non-self via binding soluble complement inhibitors, such as

factor H (FH), favouring host protection (Meri and Pangburn, 1990).

FH is a key complement inhibitor which is distributed in plasma and body fluids (Weiler et al., 1976; Whaley and Ruddy, 1976; Pangburn et al., 1977; Józsi et al., 2004). This 150 kDa glycoprotein is composed of 20 complement control protein (CCP) domains. The N-terminal part of the molecule (CCPs 1–4) is responsible for its complement regulatory activity (Alsenz et al., 1984; Kühn et al., 1995). FH has multiple binding sites for C3b, located within CCPs 1–4, CCPs 12–15 and CCPs 19–20 (Sharma and Pangburn, 1996; Jokiranta et al., 2000), and for heparin, located in CCP7, CCP9, CCPs 12–14, and CCPs 19–20 (Pangburn et al., 1991; Blackmore et al., 1996, 1998; Ormsby et al., 2006). However, in its native conformation the C-terminal domains contain the preferential interaction site for both C3b/C3d and heparin/glycosaminoglycans (Oppermann et al., 2006). Recent data have shown that FH binds to cell surfaces via its C-terminal recognition domain which is contained in CCPs 19–20 (Pangburn, 2002; Manuelian et al., 2003; Jokiranta et al., 2005; Józsi et al., 2006; Ferreira et al., 2006). This has medical relevance since FH mutations associated with atypical hemolytic uremic syndrome (aHUS) cluster in the C-terminus of the protein (Caprioli et al., 2001; Pérez-Caballero et al., 2001; Richards et al., 2001). Recombinant FH proteins which have aHUS-associated amino acid exchanges in the C-terminal CCPs 19 and 20 and patient-derived mutant FH proteins show defective binding to heparin, glycosaminoglycans, C3b/C3d and to endothelial cells (Hellwage et al., 2002; Sánchez-Corral et al., 2002, 2004; Manuelian et al., 2003; Jokiranta et al., 2005; Józsi et al., 2006). Thus, demonstrating an important role of the C-terminal region for both ligand recognition and cell binding, and suggesting that defective surface binding of FH is related to the pathology of aHUS.

Here we characterize FH activity at the host cell surface in the presence of membrane-bound complement regulators, using human umbilical vein endothelial cells (HUVEC) as a model for self-cells. We show that FH attached to these cells exerts complement regulatory activity in concert with the integral membrane regulators CD46, CD55 and CD59. This activity is, however, dependent on an intact recognition region of FH, as it is blocked by mAbs which bind to the C-terminus of the molecule. These results explain the association of C-terminal FH mutations with aHUS and have a broader relevance as they shed light on the principal mechanism of discriminating self from non-self by complement.

2. Materials and methods

2.1. Sera and monoclonal anti-FH antibodies

Normal human serum from healthy laboratory personnel was used in the experiments. FH-depleted plasma was generated by incubating human plasma in the presence of 5 mM EDTA with FH-specific antibody coupled to Sepharose beads. Recombinant FH deletion fragments were produced as described (Kühn et al., 1995). The FH blocking MH10 mAb was described (Barilla-LaBarca et al., 2002). FH-specific mAbs C21, C18, E14, E22 and H04 were generated and characterized (Oppermann et al., 2006). C1q showed background binding to these mAbs when compared with complement-fixing mAb (data not shown).

2.2. FH binding on endothelial cells

HUVEC were cultivated as described (Manuelian et al., 2003). Cells were detached by incubation in 0.02% EDTA/PBS and resuspended in cation-free PBS (DPBS). For flow cytometry, DPBS was supplemented with 1% FCS and 0.1% NaN$_3$. HUVEC (5×10^5 cells/sample) were incubated with 10 µg/ml purified FH (Merck, Schwalbach, Germany) or with diluted normal human serum in 50 µl at 37 °C for 15 min. To detect binding, mAb H04 (20 µg/ml), goat anti-human FH antiserum (diluted 1:1000) (Merck), FITC-conjugated F(ab$'$)$_2$ fragments of goat anti-mouse Ig and FITC-conjugated rabbit anti-goat Ig (both diluted 1:50) were used. All incubation steps were performed at 4 °C for 15 min. To measure the effect of mAbs on FH binding, human serum at 20% dilution was preincubated with 10 µg mAb for 5 min, then added to the cells for 15 min at 4 °C. Propidium iodide was added to stain dead cells. Forward and side scatter parameters were used to define the cell population and 10,000 cells were routinely measured using a BD LSR II flow cytometer and the FACSDiva software for data analysis (BD Biosciences, Heidelberg, Germany).

2.3. Quantification of cell-bound complement proteins

Anti-CD35 (Dako, Hamburg, Germany), anti-CD55 (Merck), anti-CD46 and anti-CD59 (BD Biosciences), anti-FH (clone H04) and isotype control (Dako) mAbs were used at saturating concentration to calculate the antibody binding capacity (ABC) for each molecule. Calibration curves were established using standard beads and antibody binding was quantified according to the supplier's instructions (QIFIKIT, Dako).

2.4. FH binding to C3b

MaxiSorp plastic plates (Nunc, Wiesbaden, Germany) were coated with 5 µg/ml C3b. Non-specific binding sites were blocked with 3% BSA in PBS. 100 ng purified FH was preincubated with 2 µg mAb for 5 min at 20 °C, then added to the wells in 50 µl PBS. FH binding was detected as described (Józsi et al., 2006).

2.5. Fluid phase cofactor assay

Five hundred nanograms FH was preincubated with 5 µg mAb in 10 µl PBS at 20 °C for 5 min. Then 0.2 µg C3b and 0.1 µg factor I (Merck) were added in 5 µl. After 10 min incubation at 37 °C, the reaction was stopped by addition of SDS sample buffer supplemented with dithiothreitol and heating at 95 °C for 5 min. Proteins were separated by 10% SDS-PAGE, blotted onto nitrocellulose membrane and developed using goat anti-human C3 (1:1000; Merck).

2.6. C3 deposition on HUVEC

Human serum diluted in DPBS to 20% was added to HUVEC and incubated at 37 °C in 50 μl. After washing, cell surface deposited C3 was detected with C3c- and C3d-specific polyclonal antibodies (both diluted 1:100) and a FITC-conjugated swine anti-rabbit Ig (1:50; Dako). For some assays the serum was preincubated with 10 μg of the blocking C18 mAb for 5 min at 20 °C. Parallel samples were lysed in DPBS containing 1% Triton X-100 and enzyme inhibitors (Roche, Mannheim, Germany). Cell extracts were separated by 10% SDS-PAGE, transferred to nitrocellulose membrane, which was developed using goat anti-human C3 antiserum (1:1000; Merck).

2.7. MAC deposition

HUVEC were incubated in human serum or in serum that was pretreated with the various FH-specific mAbs as described for the C3 measurement. In a control sample 10 mM EDTA was added to block complement activation. Cells were incubated at 37 °C for 30 min, washed thoroughly and each sample was divided into two. MAC deposition was measured with a mAb specific to C5b-9 (10 μg/ml; clone aE11, Dako) added to every second sample, followed by FITC-conjugated F(ab′)₂ fragments of goat anti-mouse Ig (1:50) added to all samples. Specific fluorescence was calculated for every sample pair by subtracting the signal obtained when cells were incubated with the sec-

ondary antibody only to eliminate data distortion due to binding of FH-specific mAbs to the cells.

2.8. Cell lysis assay

HUVEC were incubated in 10% or 20% FH-depleted plasma in 100 μl reaction volume in the presence of 20 mM Mg^{2+} to allow alternative complement pathway activation for 60 min at 37 °C. Membrane damage was detected by the release of calcein (Sigma–Aldrich, Taufkirchen, Germany; Neri et al., 2001), measured in the supernatant using a Typhoon 9410 fluorescence reader (GE Healthcare, Freiburg, Germany) set to 530 nm. The GPI-anchored regulators CD55 and CD59 were removed from the cells by treatment with PI-PLCγ (Sigma–Aldrich) for 30 min at 37 °C. Cells were incubated in FH-depleted plasma or in FH-depleted plasma which has been reconstituted with FH (50 μg/ml) and calcein release was measured as above.

3. Results

3.1. Quantification of membrane complement regulators on HUVEC

In order to assess the contribution of FH to cell surface complement control, first the presence and level of membrane-anchored complement regulators were analyzed on HUVEC by flow cytometry. These cells expressed CD46, CD55 and CD59,
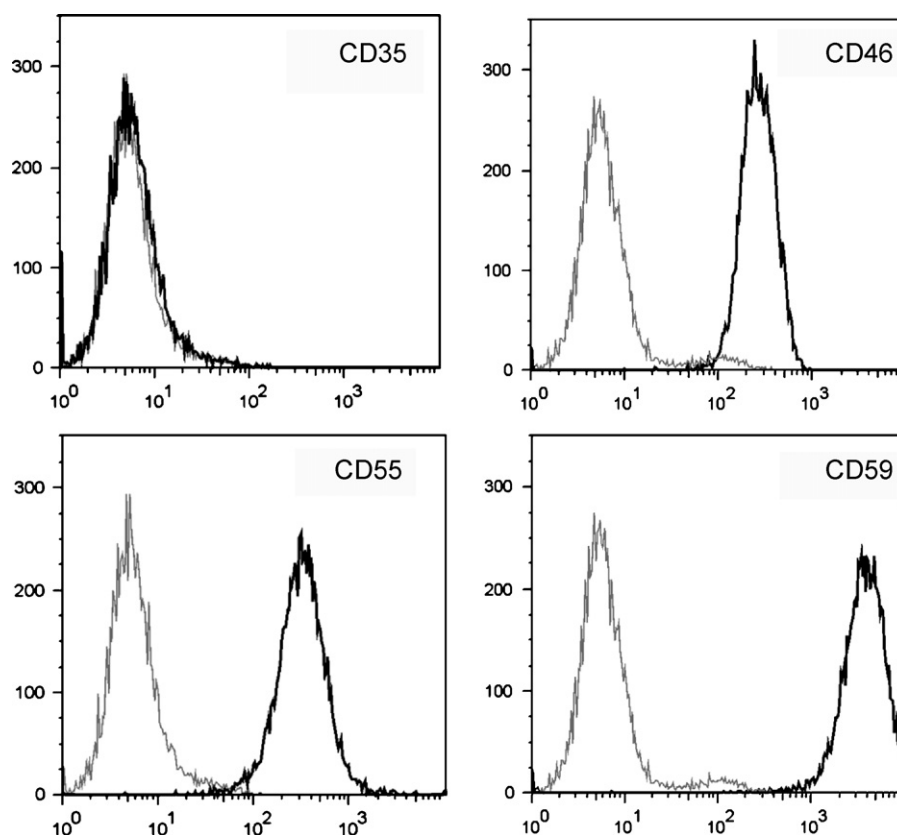

Fig. 1. Expression of complement regulators on HUVEC. The presence of CD35 (CR1), CD46 (MCP), CD55 (DAF) and CD59 was measured using specific mAbs. The x- and y-axes correspond to relative fluorescence intensity and relative cell number, respectively.

Table 1
Number of complement proteins on the surface of HUVEC

Complement protein	Number of bound proteins
CD35	Not detectable
CD46	$127,800 \pm 2280$
CD55	$178,500 \pm 51,856$
CD59	$1,602,600 \pm 357,094$
Factor H	1802 ± 798

The number of surface-bound regulators was determined using specific mAbs and calibration beads and is expressed as the specific antibody binding capacity. Data for the membrane-bound regulators represent mean $\pm$ S.D. of four experiments. The number of bound FH molecules was determined after 15 min incubation of the cells using sera from different donors in six independent experiments.

but not CD35 (Fig. 1). Using quantification beads, the approximate number of these regulators was determined. CD46 was present in ∼125,000 copies per cell. CD55 levels were about 1.5-fold higher and CD59 levels were about tenfold higher (Table 1).

3.2. FH binding to HUVEC

FH attachment to HUVEC was characterized in more detail. At low ionic strength, i.e. using 50% PBS as buffer (Manuelian et al., 2003; Jokiranta et al., 2005; Józsi et al., 2006), prominent binding of purified human FH to HUVEC was detected, which was reduced at physiological ionic strength (Fig. 2A). Thus, FH binding to endothelial cells is a weak interaction and is ionic strength dependent. A dose dependent binding which correlated with serum concentration was observed when normal human serum was used as FH source (Fig. 2B). When C3b was deposited on the cell surface by preincubating the cells with an antiserum raised against HUVEC, FH binding was enhanced (Fig. 2C). If complement activation was blocked using EDTA, abrogation of C3b-deposition was accompanied with a strong reduction of FH binding (data not shown). Thus, cell surface glycosaminoglycans and also deposited C3b mediate FH binding. The amount of cell-bound FH was calculated to ∼2000 molecules/cell using human serum as FH-source (Table 1).

3.3. Blocking of FH binding to endothelial cells

For functional analysis of cell attached FH we aimed at identifying mAbs that inhibit FH binding to the cells, but do not affect the complement regulatory activity of the protein in plasma. First the binding domain of mAb MH10, which has previously been shown to inhibit FH function on cells (Barilla-LaBarca et al., 2002; Riley-Vargas et al., 2005), was characterized. In human serum, mAb MH10 identified FH and the related FHR-1 protein (Fig. 3A, lane 1). Using recombinant deletion fragments of FH, mAb MH10 bound to CCPs 15–20 and CCPs 19–20, but not to CCPs 1–7, CCPs 8–11, CCPs 11–15, CCPs 15–18 and CCPs 15–19 (Fig. 3B). Thus, mAb MH10 binds within the C-terminal recognition region of FH, most probably to CCP20. The binding domains of MH10 and of the additional mAbs that were used in this study are shown in Fig. 3C. The mAb E22 binds to the N-terminus (CCP3) of FH, mAb C21 within CCPs 15–18, and
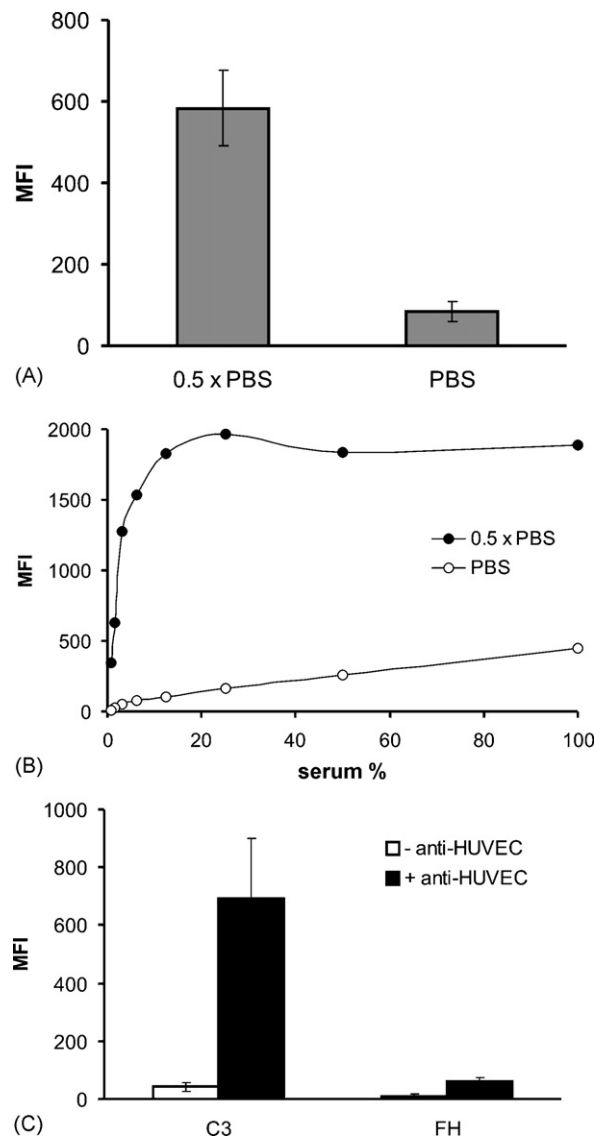

Fig. 2. FH binding to HUVEC. (A) Comparison of binding of purified FH to HUVEC in $0.5 \times$ PBS and in PBS, as determined by flow cytometry. Mean $\pm$ S.D. of median fluorescence intensities (MFI) from three experiments are shown. (B) Dose-dependent binding of FH from human serum to HUVEC in low ionic strength buffer ($0.5 \times$ PBS) and under physiological conditions (PBS), as measured by flow cytometry. (C) C3 deposition enhances FH binding to HUVEC. The cells were pre-incubated with anti-HUVEC antiserum, then exposed to 10% human serum in PBS. C3 deposition and FH binding was analyzed by flow cytometry.

mAbs C18 and E14 recognize CCP20 (Oppermann et al., 2006).

To assay how these mAbs affect FH attachment to cells, HUVEC were incubated with human serum, which had been preincubated with the indicated mAbs, and FH binding was assessed by flow cytometry. All three mAbs that bind to CCP20 of FH, i.e. C18, E14 and MH10, but not mAb C21, strongly reduced FH binding to HUVEC (Fig. 4A).

As FH binding to C3b is central for its regulatory function, the effect of the various mAbs on FH binding to immobilized C3b and the effect on fluid phase complement control were tested. The C-terminally binding mAbs C18, E14 and MH10 strongly reduced FH binding to C3b, but mAbs C21 and E22 showed
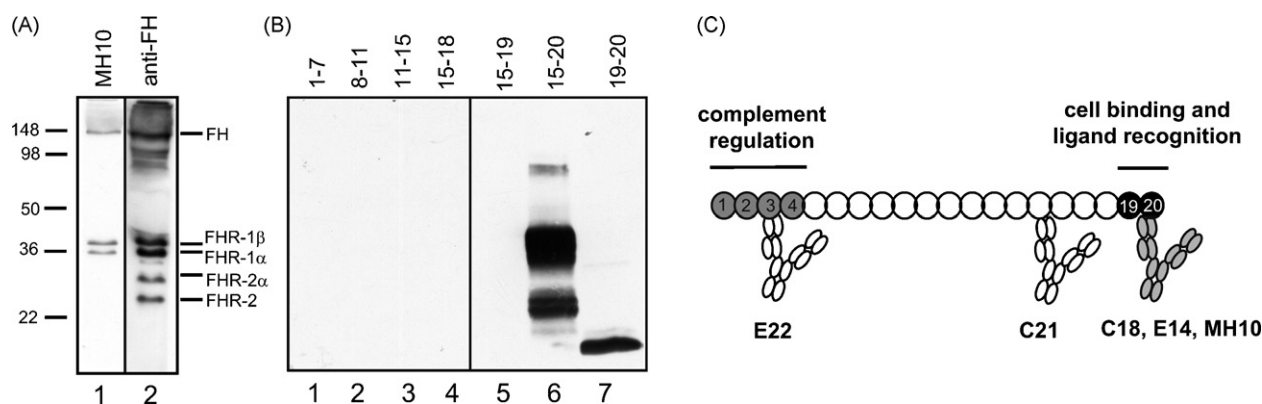
Fig. 3. Characterization of FH-binding mAbs. (A) Mapping of the binding domain of mAb MH10. Human serum was separated by SDS-PAGE, transferred to a membrane and the blot was developed with mAb MH10 (lane 1) or a polyclonal FH antiserum (lane 2). (B) Recombinant FH fragments, i.e. CCPs 1–7, 8–11, 11–15, 15–18, 15–19, 15–20 and 19–20 were separated by SDS-PAGE and analyzed by Western blotting using mAb MH10 (lanes 1–7). The upper bands in lane 6 are due to oligomerization of the FH fragment CCPs 15–20. (C) Binding sites of the mAbs used in this study. mAb E22 binds CCP3, and mAb C21 binds within CCPs 15–18 of FH. The mAbs C18, E14 and MH10 bind to the C-terminal CCP20 domain of FH.

no effect (Fig. 4B). When complement regulation was tested in fluid phase, none of the mAbs inhibited cofactor activity of FH (Fig. 4C). Apparently mAb E14 affected C3b cleavage, as in the presence of this mAb different cleavage products of the α'-chain were observed (Fig. 4C, lane 5). Thus, the C-terminally binding mAbs inhibit FH binding to cells and surface-bound C3b, but do not inhibit cofactor activity, which is mediated by the N-terminus. Since the three mAbs C18, E14 and MH10 bound to the same C-terminal domain of FH, i.e. CCP20, and had similar inhibitory effect on FH binding, these mAbs were used interchangeably throughout the experiments.

3.4. Role of FH in control of C3b deposition and cleavage on the surface of endothelial cells

Complement activation on the surface of endothelial cells was analyzed by following C3b deposition and processing with polyclonal antibodies, which are specific for either the C3c or the C3d fragments. The anti-C3c antibody reacts with C3b and iC3b, but not with C3d, which is the final cleavage product and remains covalently bound to the cell membrane. By contrast, the anti-C3d antibody identifies all forms of surface-bound C3b. These two antibodies allowed determination of cell bound C3c:C3d ratio over time, which reflects deposition and fragmentation of C3b on the cell surface.

Upon exposure to human serum, the amount of deposited C3b on HUVEC increased during the first minutes (Fig. 5A). The C3c-specific antibody identified more epitopes than the C3d-specific antibody, therefore the C3c:C3d ratio was higher than 1 (Fig. 5B, start). Continuous processing and inactivation of surface-bound C3b was shown by the higher increase in the C3d-fluorescence as compared to the C3c-fluorescence (Fig. 5A and B).

Inactivation of C3b results in reduction of C3c-reactivity, and a reduction of the C3c/C3d ratio. In a normal situation, when the cells are exposed to serum, this degradation occurs within minutes (Fig. 5B, open circles). In order to assay the role of surface-attached FH in this process, FH binding was inhibited with the C-terminally binding mAb C18. Blocking of FH binding

affected the kinetics of C3b degradation, as shown by the higher C3c/C3d ratio (Fig. 5B, filled circles). This was due to higher reactivity of the C3c-specific antibody, which reflected increased number of intact C3b molecules and a lower rate of C3b degradation at the cell surface. This effect was pronounced during the initial phase, but diminished at later times. The observed inhibitory effect during the early phase of the reaction was confirmed using other mAbs (Fig. 5C).

In addition, C3b inactivation on the cell surface was visualized directly by Western blotting. For cells incubated with serum, complete cleavage of the α'-chain of C3b was detected (Fig. 5D, lane 3). However when FH binding was blocked, inactivation of C3b was slower and the α'-chain of C3b was still present (Fig. 5D, lane 4). In summary, these results demonstrate a role in complement regulation for FH on the surface of HUVEC.

3.5. FH affects MAC formation on endothelial cells

C3b deposition and complement activation on HUVEC was also analyzed using FH-depleted plasma. Under these conditions, low FH binding and high level of C3b deposition was observed (Fig. 6A, empty bars). Upon reconstituting the plasma with purified FH, FH binding was enhanced, which was accompanied with lower amount of surface deposited C3b and MAC (Fig. 6A, filled bars).

The role of surface-attached FH on MAC formation was also studied using mAbs that block FH binding. When cells were incubated in normal human serum, MAC deposition was observed to a relatively low extent (Fig. 6B). However, the number of surface deposited MAC was increased when FH binding was blocked by mAbs C18, E14 or MH10. This effect was due to reduced FH binding to HUVEC as mAb C21 showed no effect (Fig. 6B). When complement activation was blocked with EDTA, no MAC was detected (Fig. 6B). This relatively low effect of cell attached FH on MAC formation in contrast to the higher impact on complement activation at the C3-level was indicative of the role of the membrane regulators in limiting MAC formation.
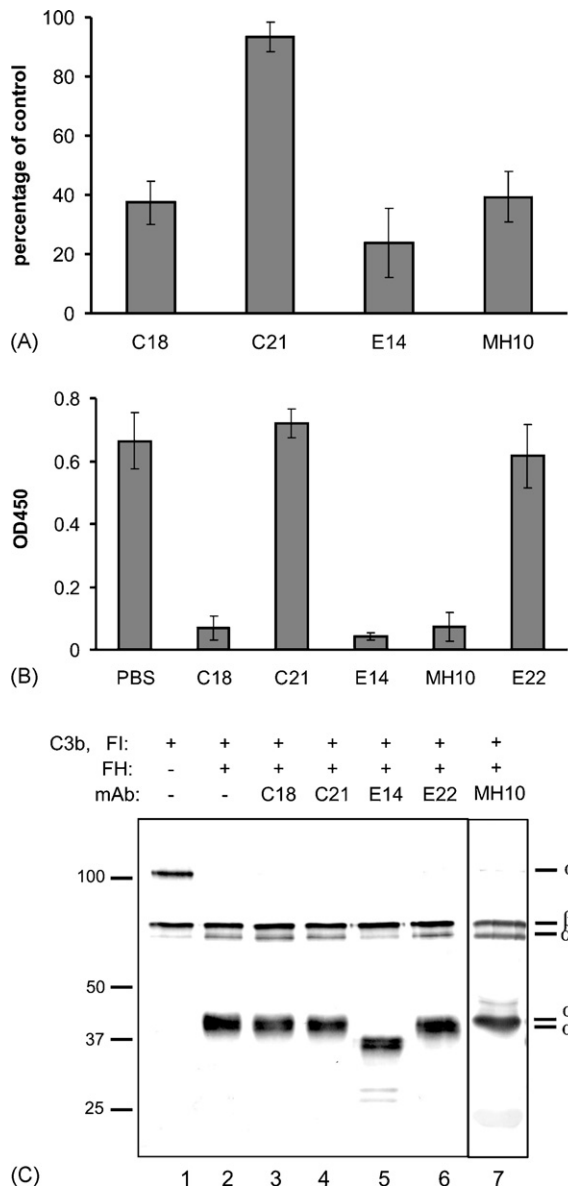
Fig. 4. Effect of mAbs on FH functions. (A) Binding of FH to HUVEC is reduced by the C-terminally binding mAbs C18, E14 and MH10, but not by mAb C21, as determined by flow cytometry. Human serum preincubated with the indicated mAbs was added to the cells. FH binding to the cells in the absence of mAb was set to 100% and the inhibitory effect of the various antibodies is shown (mean ± S.D. of five experiments). (B) FH binding to immobilized C3b is inhibited by C-terminally binding mAbs. FH preincubated with the indicated antibodies was assayed for binding to C3b by ELISA. Mean ± S.D. of data from four experiments is shown. (C) The FH-binding mAbs do not inhibit FH-mediated cofactor activity in fluid phase. C3b was incubated with factor I in the presence of FH pretreated with the indicated mAbs. After 10 min, the samples were separated by SDS-PAGE under reducing conditions, and C3-fragments were identified by Western blotting.

3.6. Cooperation of FH and membrane regulators to prevent lysis of endothelial cells

Although blocking of FH binding by mAbs resulted in increased MAC deposition, mAbs C18, E14 and MH10 did not cause a significant increase in HUVEC lysis measured after 1 h (data not shown). When HUVEC were incubated in

FH-depleted plasma, cell lysis was dependent on the plasma concentration (Fig. 7A). In order to minimize the contribution of the integral membrane bound regulators, the GPI-anchored surface proteins were removed from the cell surface by treatment with PI-PLCγ. After this enzymatic treatment ∼25% of the CD55 and CD59 molecules remained surface bound, as determined by flow cytometric analysis (data not shown). When these CD55^{low}/CD59^{low} cells were exposed to FH-depleted plasma, cell lysis was enhanced compared to the untreated cells (Fig. 7B). Upon reconstitution of the depleted plasma with FH, cell lysis was reduced to background level, demonstrating the cooperation of FH and membrane regulators to inhibit complement activation at the cell surface (Fig. 7B, filled bar).

4. Discussion

Discriminating self from foreign surfaces is a major task of the complement system, which in this recognition process relies on self-associated molecular markers, such as cell surface glycosaminoglycans (Meri and Pangburn, 1990). In the present report we provide evidence that complement regulation on host cells and tissues largely depends on an intact C-terminus of FH, which allows surface binding and the consequent surface-associated regulatory activity of FH.

FH binding to various cells have been described, and certain disease situations, such as the kidney disease hemolytic uremic syndrome, have indicated an important role for the carboxyl-terminus of FH in host protection (reviewed in Józsi et al., 2004). Homology-based molecular models of CCPs 19–20 have shown that amino acid residues mutated in aHUS patients may affect structure of these domains or they are involved in FH binding to heparin and C3b (Hellwage et al., 2002; Perkins and Goodship, 2002; Józsi et al., 2006). Two recent reports provide insight into the structure of CCPs 19–20. An NMR-based study confirmed the role of mutated residues in either maintaining domain structure or heparin binding (Herbert et al., 2006). In contrast to this, from the X-ray structure of the C-terminal domains a disturbance of the interaction between FH and C3b was concluded for most of the surface-exposed aHUS-associated mutations (Jokiranta et al., 2006). Despite these evidences, since host cells usually express various membrane complement inhibitors, the role of FH as a cell surface regulator has not been fully accepted. Using HUVEC as a model of nucleated host cells, we demonstrate that both FH and cell membrane regulators are required for proper protection from complement mediated damage.

The significance of functionally active surface-attached FH in complement regulation at the cell surface was shown by (i) inhibiting FH binding to endothelial cells with domain mapped mAbs, which however did not affect FH complement regulatory activity in fluid phase (Figs. 4–6); (ii) using FH depleted, but complement active plasma (Figs. 6 and 7); (iii) assaying complement mediated cell lysis after enzymatic removal of the GPI-anchored membrane regulators CD55 and CD59 (Fig. 7). All three scenarios showed that, in the absence of cell bound FH, C3 deposition, MAC formation and/or complement mediated cell lysis was increased.
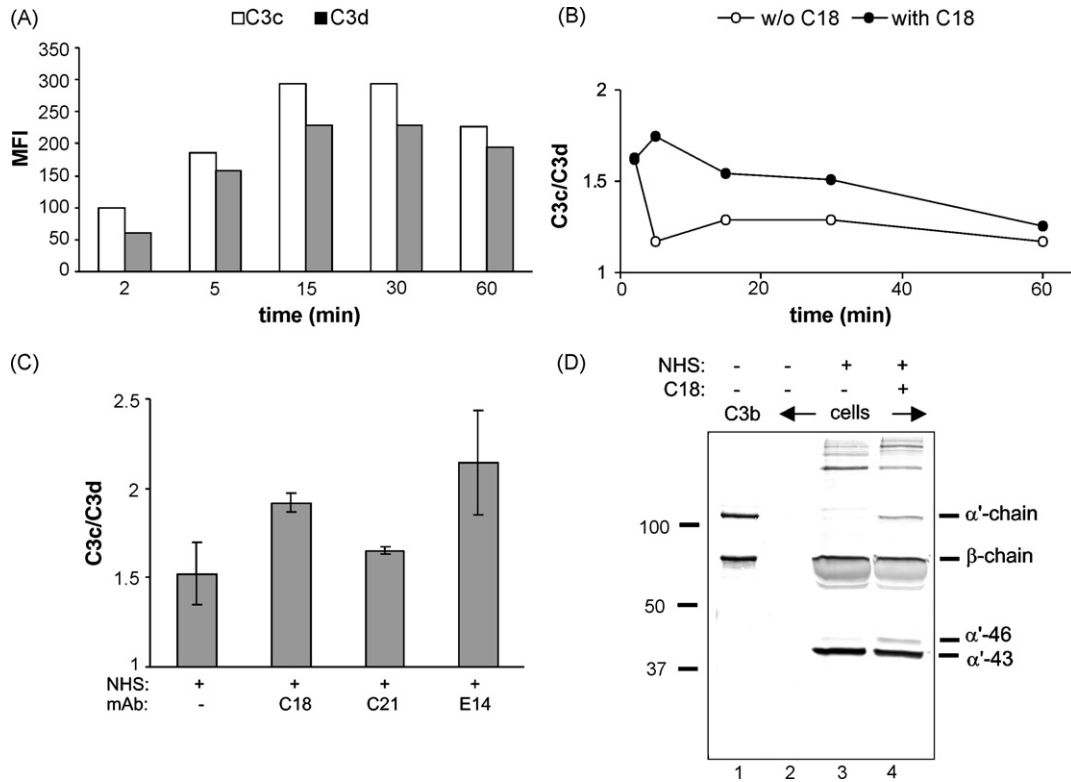
Fig. 5. Surface deposition and fragmentation of C3b on HUVEC. (A) HUVEC were incubated for the indicated time with human serum, stained with C3c- and C3d-specific antibodies and analyzed by flow cytometry. The C3c-antibody detects C3b and iC3b, but not the final C3d fragment. The C3d-antibody detects the C3d domain, which is present in all types of surface-bound C3b-fragments. Data of a representative experiment are shown as median fluorescence intensities (MFI). (B) HUVEC were incubated with human serum in the absence (open circles) or presence (filled circles) of mAb C18, which blocks FH binding. C3-fragments were analyzed by flow cytometry. The ratio of C3c- and C3d-specific fluorescence values was calculated at the indicated time points. (C) Effect of the various FH binding mAbs on the fate of C3. The C3c/C3d ratio was determined after incubating the cells for 5 min as above. Means ± S.D. of data from four experiments are shown. (D) Blocking FH attachment affects C3 degradation on the cell surface. HUVEC were incubated for 5 min in human serum which has been pretreated with mAb C18. Cell lysate was separated by SDS-PAGE and analyzed by Western blotting using a C3-specific antiserum. Lane 1, purified C3b is shown to indicate mobility of the α′- and β-chains; lane 2, lysate prepared from HUVEC incubated in PBS; lane 3, cells incubated in human serum; lane 4, cells incubated in serum in the presence of mAb C18. The high molecular weight bands in lanes 3 and 4 represent α′-chain fragments covalently bound to membrane molecules.

Upregulation of FH expression and FH binding to cells and tissues have been reported in several disease settings where inflammation and complement activation is involved, e.g. for synovial fibroblasts in rheumatoid arthritis (Friese et al., 2000, 2003), for atherosclerotic lesions (Oksjoki et al., 2003) and for tumor cells (Junnikkala et al., 2000, 2002). FH polymorphisms, mutations and deficiency have been linked to age-related macular degeneration, aHUS and membranoproliferative glomerulonephritis (reviewed in Zipfel et al., 2006). FH acts as regulator on melanoma cells during classical pathway activation (Ollert et al., 1995) and on apoptotic cells (Gershov et al., 2000). On CD46-transfected CHO cells FH was the major cofactor for C3b-cleavage, as demonstrated with the FH-blocking MH10 antibody (Barilla-LaBarca et al., 2002). On acrosome-reacted
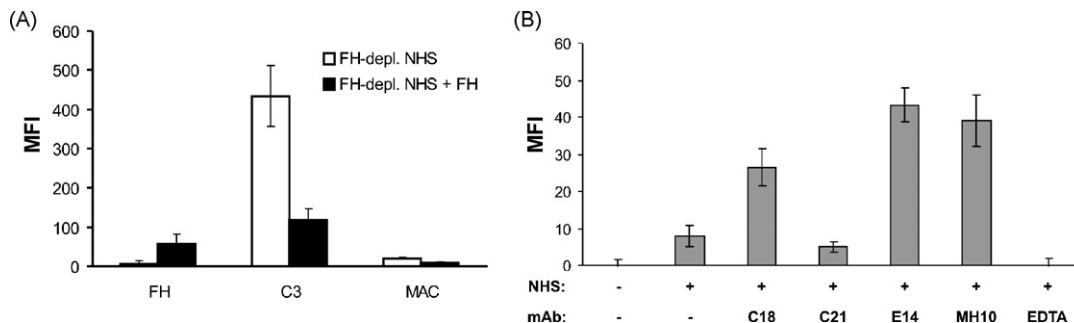

Fig. 6. Role of FH in C3b and MAC deposition on HUVEC. (A) HUVEC were incubated in 10% FH-depleted human plasma (empty bars) and FH binding, C3b deposition and MAC formation were analyzed by flow cytometry. Reconstitution of the FH-depleted plasma with purified FH resulted in reduced C3b and MAC deposition (filled bars). (B) HUVEC were incubated in 20% normal human serum (NHS) in the absence or presence of the indicated mAbs or in EDTA. MAC formation was detected on the cell surface by flow cytometry using a C5b-9-specific mAb. The mean of specific median fluorescence intensities (MFI) ± S.D. are shown from four independent experiments.
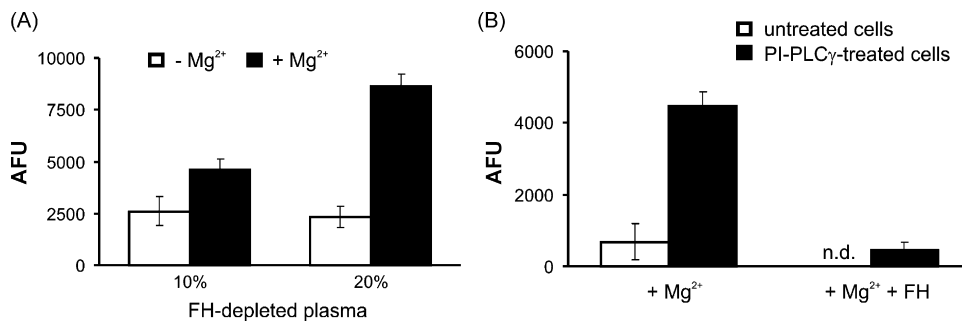
Fig. 7. Role of FH in complement mediated cell lysis. (A) HUVEC were incubated with FH-depleted human plasma in the absence (empty bars) or presence (filled bars) of Mg^{2+} for 60 min. Cell lysis as assayed by calcein release is shown in arbitrary fluorescence units (AFU). (B) FH protects HUVEC from complement mediated lysis. Incubation of the cells in FH-depleted plasma resulted in low lysis as indicated by the release of calcein (empty bar). Following enzymatic removal of the membrane regulators CD55 and CD59, complement mediated lysis was increased (left panel, filled bar). The protective role of FH is demonstrated when FH-depleted plasma was reconstituted with purified FH (right panel, filled bar). The values of FH-depleted plasma without Mg^{2+} added was subtracted as negative control. n.d., not determined.

spermatozoa, which express CD46, FH assisted C3b-cleavage by factor I as shown by the inhibitory effect of mAb MH10 (Riley-Vargas et al., 2005). Here we mapped the binding epitope of this mAb to the C-terminal recognition and cell binding region of FH (Fig. 3), showing that in these studies FH activity was actually dependent on its C-terminus, which supports our findings.

Thus, both of the two separated major functional regions of FH, namely, the N-terminal domains mediating cofactor/decay accelerating activity, and the C-terminal domains (CCPs 19–20) mediating polyanion as well as C3b binding, are required for FH activity on cell and tissue surfaces, whereas the N-terminal regulatory domains are sufficient for FH activity in plasma. This difference is also reflected in two distinct kidney diseases. The missing activity of the N-terminal domains caused by homozygous FH deficiency (Ault et al., 1997; Dragon-Durey et al., 2004), N-terminally binding autoantibody (Jokiranta et al., 1999) or by mutation in CCP4 (Licht et al., 2006) leads to insufficient complement regulation both in plasma and on tissue surfaces, and manifest as membranoproliferative glomerulonephritis. By contrast, a defective or missing C-terminus of FH caused by heterozygous mutations allows proper FH activity in plasma, but reduces cell surface protection, e.g. of endothelial cells, and is associated with aHUS. Endothelial cells are sensitized with active C3-fragments in the absence of FH (Fig. 5), increasing the susceptibility of the cells to complement-mediated damage. Cell lysis, however, is limited by the integral complement regulators (Fig. 7), in particular by the terminal regulator CD59, which is expressed at high levels on HUVEC (Table 1). It is likely that during excessive complement activation the maximal activity of both membrane anchored and surface-attached regulators, such as FH, is required. This is in line with the current concept of aHUS that considers FH and defective complement control as predisposing factors for the disease (Noris and Remuzzi, 2005), and is consistent with reports demonstrating that mutations in other regulators, such as MCP and factor I, also predispose to aHUS (Richards et al., 2003; Noris et al., 2003; Fremeaux-Bacchi et al., 2004; Kavanagh et al., 2005).

Glycosaminoglycans allow low affinity binding of FH to the cell surface (Fig. 2 and Jokiranta et al., 2005). Since the efficiency of the complement system is built on its amplifying activation mechanism, the relatively low number of surface attached FH (~2000 molecules per cell), as compared to the membrane regulators (Fig. 1 and Table 1), is sufficient to attenuate complement activity on the cells at an early phase (Fig. 5). Even if the initial complement control by FH is inappropriate, the membrane regulators can at least partially compensate and prevent direct lysis (Fig. 7).

Polyanionic structures, i.e. sialic acid and various glycosaminoglycans, are present on a broad range of self cells and tissues, therefore, surface associated activity of FH has a general relevance for host protection against complement mediated damage. The necessity of these negatively charged host cell marker molecules for FH in inactivation of deposited C3b is also indicated by the observation that rabbit erythrocytes, which have very low amount of sialic acid residues, are readily lysed in human serum (data not shown and Pangburn, 2002). By contrast, sheep red blood cells (SRBC), which have a surface rich in sialic acids similar to human cells, are relatively resistant to human complement, because they allow binding of FH. When FH binding was blocked with C-terminally binding mAbs, SRBC lysis was markedly enhanced. Human erythrocytes, which display both glycosaminoglycans and membrane regulators (CD35, CD55 and CD59) on their surface, could not be lysed under the same conditions (data not shown). Thus, the number and combination of membrane regulators and polyanionic surface marker molecules expressed by individual cells determine their resistance to complement mediated damage. A study published recently reached similar conclusion (Ferreira et al., 2006). Using recombinant FH CCPs 19–20 fragments, FH binding and surface activity could be blocked, resulting in increased lysis of SRBC. Efficient lysis of human erythrocytes, however, required blocking of CD55 or CD59 in addition.

In summary, the data allow assessment of FH activity in relation to cell membrane regulators on surfaces, provide insight into the mechanism of self/non-self differentiation and host cell protection, and point to a principal role of the FH C-terminus in this fundamental innate immune function.

Acknowledgements

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