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Complement factor I in health and disease

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Abbreviations: aHUS, atypical hemolytic uremic syndrome; C4BP, C4b-binding protein; CFI, Complement factor I gene; CR1, complement receptor 1; DFP, diisopropylfluorophosphate; ESRD, End-stage renal disease; FH, Factor H; FI, Factor I; FIMAC, factor I membrane attack complex (domain); HUVEC, human umbilical vein endothelial cells; Inr element, Initiation element; LDLr, low density lipoprotein receptor domain; SP, serine protease domain; MCP, membrane cofactor protein (CD46); NHS, normal human serum

Abstract

Factor I (FI) is a crucial inhibitor controlling all complement pathways due to its ability to degrade activated complement proteins C3b and C4b in the presence of cofactors such as factor H, C4b-binding protein, complement receptor 1 or CD46. Complete deficiency of FI, which is synthesized mainly in the liver is rare and leads to complement consumption resulting in recurrent severe infections, glomerulonephritis or autoimmune diseases. Incomplete FI deficiency is in turn associated with atypical haemolytic uremic syndrome, a severe disease characterized by thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure. Structurally, FI is a 88 kDa heterodimer of a heavy chain consisting of one FI-membrane attack complex (FIMAC) domain, one CD5 domain and two low-density lipoprotein receptor domains (LDLr), and a light chain which is a serine protease domain (SP), linked to the heavy chain by a disulfide bond. FI cleaves its *in vivo* substrates C3b and C4b only in the presence of cofactors, it shows poor enzymatic activity towards synthetic substrates tested so far and it has no natural inhibitor.

1. Introduction

The complement system plays a major role in defense against pathogens. It also identifies dying cells, immune complexes or misfolded molecules (Ricklin et al., 2009) and guides adaptive immunity (Markiewski and Lambris, 2007). The physiological relevance of complement is demonstrated by illnesses affecting complement deficient patients such as recurrent infections, autoimmune diseases and kidney diseases (Pettigrew et al., 2009; Welch and Blystone, 2009). Invading pathogens activate complement either spontaneously, due to differences in surface composition compared to host cells, or through antibody or pentraxin binding (Lambris et al., 2008). This leads to rapid initiation of a proteolytic complement cascade, release of pro-inflammatory anaphylatoxins that influence blood vessel permeability (C5a, C3a) and attract white blood cells (C5a), opsonisation of the target with C3b and finally formation of the membrane attack complex (MAC).

Complement has to be tightly regulated by both soluble and membrane bound regulators to protect self-tissues from complement-mediated damage (Sjoberg et al., 2009). Many of these inhibitors are located in chromosome 1q32 and they are collectively termed regulators of complement activation (RCA). The RCA proteins inhibit the complement system by accelerating the decay of the C3 and C5 convertases and/or by acting as a cofactor for a serine proteinase factor I (FI) in the degradation of C3b and C4b. FI inhibits all the pathways of complement by cleaving the α'-chain of the activated C3b and C4b proteins. This, however, can only occur in the presence of the following cofactors: factor H (FH), C4b-binding protein (C4BP), membrane cofactor protein (MCP; CD46) or complement receptor 1 (CR1; CD35).

2. FI

FI is an 88 kDa serum glycoprotein that is expressed in the liver by hepatocytes (Morris et al., 1982), but also by other cells such as monocytes (Whaley, 1980), fibroblasts (Vyse et al., 1996), keratinocytes (Timar et al., 2007) and human umbilical vein endothelial cells (HUVEC) (Julen et al., 1992). The average serum concentration of FI is ~ 35 μg/ml and increases during inflammation since FI is an acute phase protein. Thus, the expression of factor I is upregulated by IL-6 in hepatocytes (Minta et al., 1998; Morris et al., 1982) and by interferon-γ (IFN-γ) in keratinocytes (Timar et al., 2007) and HUVEC (Julen et al., 1992). FI is also expressed by some cancer cells such as glioma cells (Gasque et al., 1992) or non small cell lung cancer cells (Okroj et al., 2008), which may be contributing to protection of these altered cells from complement attack.

The promoter region upstream of the *CFI* gene is not well characterized. The human *CFI* gene lacks a TATA box but it contains a TATA like sequence (AATA) -54 to -57 upstream of the start codon (ATG) +1. Since the *CFI* gene has a TATA-less promoter an initiator element (*Inr*, TCAGCCA) is essential for the promoter activity. This *Inr* element (-148 to -154), which encompasses the transcription start point (tsp, -152), mediates the positioning of the basal transcriptional machinery (Paramaswara and Minta, 1999). However, mutation of the *Inr* site in the *CFI* gene did not completely inhibit promoter activity suggesting utilisation of adjacent regulatory element(s) for efficient transcription (Paramaswara and Minta, 1999). Furthermore, a CTGGAG(G/T) sequence has been identified in several genes to be essential for efficient initiation of transcription and necessary for binding of nuclear proteins. In the *CFI* promoter, this repeat is found twice, just upstream (-156 to -161) of the *Inr* element and downstream the TATA-like sequence (-47 to -52). Mutations in the CTGGAT site upstream of the *Inr* element reduced the promoter activity by 50% and mutations in both this sequence

and the *Inr* element almost entirely abolished the promoter activity (Paramaswara and Minta, 1999). So far, not much is known about the transcription factors needed for expression of the *CFI* gene. The CTGGAT motif and the *Inr* element play an important role in the constitutive expression of *CFI*. It is possible that this region facilitates assembly of the transcription machinery on the *Inr* site and thereby initiates transcription.

The *CFI* gene encompasses 63 kb, and it is located on chromosome 4q25 (Shiang et al., 1989). *CFI* contains 13 exons with a very large intron (35 kb) between exon 1 and 2 (Vyse et al., 1994). The exon arrangement corresponds well to the domain structure of the protein (Figure 1).

FI is a multidomain protein synthesized as a single polypeptide chain but four positively charged amino acids (RRKR) are then cleaved out to yield the heavy (50 kDa) and the light (38 kDa) chains (Goldberger et al., 1984). These chains are covalently linked via a disulfide bridge (Fearon, 1977). The protein also undergoes glycosylation and each chain contains three *N*-linked glycans. Once the protein is secreted, the signal peptide of 18 residues is cleaved off. FI can be expressed recombinantly in mammalian or insect cells but only partial proteolytic processing of the single chain FI precursor occurs under such conditions and only the processed protein has catalytic activity (Nilsson et al., 2007; Ullman et al., 1998; Wong et al., 1995)**.** Mature FI is made up of an unique sequence of domains some of which share sequence similarity with domains found in complement and non-complement proteins. The heavy chain consists of the FI membrane attack complex domain (FIMAC), a CD5-like domain (also known as the scavenger receptor cysteine-rich domain), low-density lipoprotein receptor 1 and 2 domains (LDLr1 and 2) and a small region of unknown homology, sometimes called the D-region (Figure 1). FIMAC and LDLr domains are also found in complement components C6 and C7. The light chain of FI comprises the serine protease (SP) domain (Chamberlain et al., 1998) where the catalytic triad in the active site is formed by His362, Asp411 and Ser507. FI contains 40 cysteines and 36 of them are involved in intra-domain disulfide bridges, and the remaining four cysteines (C15-C237, C309-C435) connect the FIMAC and LDLr1 domains and the SP domain with the heavy chain (Tsiftsoglou et al., 2005).

3. **Specificity and enzymology of FI**

FI (EC 3.4.21.45) was detected in the late 1960s as an activity, which altered the properties of C3b deposited on red blood cells. At an early stage it was named Konglutinogen-Activating Factor (KAF), an activity which caused complement-reacted erythrocytes to be agglutinated by a bovine protein, conglutinin (Lachmann and Muller-Eberhard, 1968). This reaction was subsequently shown to correspond to the proteolytic cleavage of C3b deposited on the erythrocytes to form iC3b, in which a high-mannose oligosaccharide is oriented to bind conglutinin, a large multivalent collectin, which cross-links the cells by binding to iC3b on different cells. At later stages FI was shown to cleave C3b and C4b, and was known in the 1970s as C3b inactivator, C4b inactivator or C3b/C4b inactivator. With improvement in protein purification methods, it was shown that more highly purified FI does not cleave C3b or C4b: an additional protein, a cofactor, was required, which formed a complex with C3b or C4b, and only the complex was attacked by FI. The soluble cofactors FH and C4BP were isolated from plasma in the 1970s, and the membrane-bound cofactors CR1 and MCP in the 1980s (summarised by (Crossley, 1981; Sim et al., 1993). Additional virus-encoded proteins, which mimic activities of the cofactor proteins were identified in vaccinia virus, herpes virus 8 and in the smallpox virus (Blom et al., 2007).

Studies on the polypeptide chain structure, and N-terminal amino acid sequence of isolated FI led to the conclusion that it was a serine protease but with atypical mode of action as it was not inhibited by the wide-spectrum serine protease inhibitor, DFP (di-isopropyl fluorophosphate) (Crossley, 1981). Complete cDNA sequencing (Catterall et al., 1987; Goldberger et al., 1987) confirmed that FI had a typical serine protease domain, related to that of trypsin, with the conserved catalytic triad, Asp-Ser-His.

Nearly all serine proteases in plasma circulate as inactive proenzymes, which become activated by proteolytic cleavage at the N-terminus of the SP domain on exposure to some trigger. Coagulation factors II, VII, IX, X, XI, XII, and other complement proteases (C1r, C1s MASPs) conform to this pattern. Once activated, they are quite rapidly inactivated by endogenous protease inhibitors such as α2-macroglobulin or the serpins. FI however, and the complement protease Factor D are unusual in that they circulate in a form which is already cleaved at the N-terminus of the SP domain, and so they would be expected to be active and to be regulated by inhibitors. However, FI and Factor D do not react with any endogenous protease inhibitors, and their enzymic activity, as assessed by use of low molecular weight synthetic substrates, is very low. However for these proteases, their substrates are transiently-formed complexes (C3b- or C4b-cofactor complexes for FI, C3b-Factor B for Factor D), and so their activity *in vivo* is regulated by supply of substrate, without need for an endogenous inhibitor. Why, however, do they appear to have such low activity? For interaction between C3b and factors D and B it has been shown that their catalytic sites are not in an active configuration, and binding to a large protein complex substrate is needed to induce an active conformation (Laursen et al., 2011; Rooijakkers et al., 2009). For FI, it has also been suggested that within the FIMAC domain, there is a subdomain resembling a protease inhibitor of the follistatin class, so that the FI active site might be inhibited by the follistatin subdomain, until binding to a substrate complex caused conformational change in FI (Ullman and Perkins, 1997).

FI does not cleave any of the chromogenic synthetic substrates developed in the 1970s-1980s for the assay of coagulation proteases or C1s. These are generally amino-acid or peptide esters or amides which generate p-nitrophenol or p-nitroaniline when cleaved. Use of more sensitive fluorescent substrates was required to demonstrate that isolated FI does have activity. Isolated native FI, in the absence of cofactors, cleaves peptide aminomethyl coumarin (AMC) substrates (Tsiftsoglou et al., 2005). Only a limited range of substrates was explored, and DPR-AMC (*tert*-butyloxycarbonyl-Asp(O-Benzyl)-Pro-Arg-AMC) and VPR-AMC (*tert*-butyloxycarbonyl-Val-Pro-Arg-AMC were the best of 15 substrates tested. The SP-domain of FI (formed by proteolysis and attached to a 20-residue remnant of the C-terminus of the heavy chain) cleaved the same substrates at a similar rate, showing that both the heavy chain, and the cofactor (FH) do not influence the cleavage of low molecular weight synthetic substrates (Tsiftsoglou and Sim, 2004). Furthermore, the SP domain of FI expressed alone cleaved GGR-r110, AR-r110, *tert*- butyloxycarbonyl-LGR-r110 and VPR-r110 (Malhotra et al., 2003).

The bonds cleaved by FI in its natural substrates are, in C3b, LPSR-SS and SLLR-SE; in iC3b, RLGR-EG; and in C4b, HRGR-TL and STGR-NG. In each case, cleavage is after the R (Arg) residue. So far, only a limited range of synthetic substrates has been tested, and none of these represent the natural substrate sequences, so the synthetic substrates do not give much useful comparison with the "real" substrates. Comparing with the RLGR-EG sequence in iC3b, GR-AMC and LGR-AMC are not substrates, but *tert*-butyloxycarbonyl-LGR-r110 is. For the C3b sites, LPSR-SV, PSR-AMC has not been tested, but the similar FSR-AMC is hydrolysed at a low rate.

Substrates based on RGR or TGR have not yet been tested. The BRENDA database (Scheer et al., 2011) summarises available data on substrates and inhibitors of FI (http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.4.21.45). The cutDB database (Igarashi et al., 2007) summarises data on cleavage sequences in natural substrates (http://cutdb.burnham.org/).

Compared with assays of FI proteolytic activity, which are done by assessing, by SDS-PAGE analysis, the rate of conversion of C3b to iC3b in the presence of FH, the synthetic substrates do allow much more rapid assay of FI amidolytic activity, and give the opportunity to test the enzymatic properties of FI, compare it with other serine proteases, and look for inhibitors, without the complication that the inhibitor may be acting on cofactors and protein substrates. Such sensitive assays have provided a useful route to investigating the activity of FI and FI variants.

Several papers on the pharmaceutical development of relatively specific inhibitors for thrombin have shown that such inhibitors also act on FI, and so FI is often regarded as thrombin-like. Both enzymes cleave at arginyl sites. However when compared with thrombin, FI is a very poor catalyst. For the substrates DPR-AMC, VPR-AMC, and FGR-AMC, the Km values are quite similar to those of thrombin (in the range 15-130 μM), but the rate of cleavage (Vmax) is ~ 5000-60000 times slower with FI (Tsiftsoglou and Sim, 2004). The most likely explanation for this is that a given time-point, only a small proportion of molecules (<< 1%) within the FI population, has an active site in the correct configuration for catalysis. The role of the cofactor (and the large protein substrate) therefore may be to induce the catalytic conformation in a larger population of FI. It is very difficult to measure a Vmax for the cleavage of C3b in the presence of FH, but a single report suggests a Vmax about 900-1000-fold higher than seen with the synthetic substrates (Pangburn and Muller-Eberhard, 1983). This would bring FI into a range of catalysis only ~5-60-fold slower than thrombin.

Assays with synthetic substrates have allowed clearer identification of some inhibitors of FI. Few relatively specific inhibitors are available for experimental use. Leupeptin (N-acetyl-L-leucyl-L-leucyl-L-argininal) and antipain (carboxy-2-phenylethyl]-carbamoyl-L-arg-L-val-argininal) are good competitive inhibitors. These resemble respectively the C3b cleavage site SLLR-SE and the C4b site, HRGR-TL. Pefabloc-SC (4-(2-Aminoethyl)-benzenesulfonyl fluoride) (irreversible), and Pefabloc-Xa (Nalpha-Tosylglycyl-3-amidino- (D,L)-phenylalanine methylester) (competitive) are also very effective inhibitors (Tsiftsoglou and Sim, 2004).

As noted above, several attempts at pharmaceutical development of thrombin inhibitors have resulted in inhibitors, which also act on FI (Fevig et al., 1998; Rupin et al., 1997). Among these are boropeptide inhibitors (Fevig et al., 1998; Tsiftsoglou and Sim, 2004). An orally bioavailable factor Xa inhibitor, DPC423, also acts on FI, although the Ki values differ by ~300000-fold (0.15 nM for Xa; 44 μM for FI; Wong et al, 2002). Other inhibitors of FI are listed on the Brenda database.

Further key data on FI is available in the MEROPS database (Rawlings et al., 2010), where FI is listed as S01.199: that is, a member of the S1 Peptidase family of serine endopeptidases (http://merops.sanger.ac.uk/cgi-bin/pepsum?mid=S01.199). The Merops database contains sequence comparisons for FI homologues and paralogues from more than 30 animal species, lists of ESTs, key literature, and data on natural substrates.

Many aspects of the interaction of FI with its cofactors FH, C4BP, MCP and CR1 have not yet been systematically studied. In forming the complex between FI, FH and C3b, each protein binds independently to the two others (ie FI-FH and FI-C3b complexes are formed, as well as FH-C3b). The binding affinities are very low, and the

interactions are mostly ionic, and therefore much easier to observe in non-physiological low-salt conditions (DiScipio, 1992; Soames and Sim, 1997). It is not known, for example, whether the two cleavages of C4b, or the 2-3 cleavages of C3b can occur sequentially within one complex, or whether this requires dissociation and reformation of the complex in a different orientation. In contrast to the activity of FI on synthetic substrates, the pH optimum of cleavage of C3b or C4b by FI in the presence of FH or C4BP is low (pH 5-6) (Seya and Nagasawa, 1985; Sim et al., 1981). This appears to be an effect of the cofactor on FI, and it is not clear what is the physiological significance of this phenomenon. However, 3D high-resolution structural details of complex formation (between C3b and cofactors) are becoming available (Wu et al., 2009) and details for FI-C3b and FI-cofactor will be available soon. Regarding the specificity of cofactors, it is generally accepted that FH acts on C3b, and C4BP on C4b, while CRI and MCP act on both. However, FH can act as a cofactor for C4b breakdown, although the activity is seen only at low salt strength and low pH (Seya et al., 1995). C4BP, similarly can act as cofactor for C3b breakdown (Blom et al., 2003; Seya et al., 1995; Sim et al., 1981).

4. Structural investigations of FI

In recent years significant efforts have been made to elucidate the binding sites for C3b and C4b on various cofactor proteins such as C4BP (Blom et al., 2001; Blom et al., 2003), CR1 (Krych et al., 1998), MCP (Adams et al., 1991), and FH (Wu et al., 2009). However, the mechanism by which the cofactors assist in the cleavage of C3b/C4b FI is far from clear. The substrates themselves may induce a conformational change in the FI protein that would make the active site accessible and would allow degradation of the substrate. In support of this hypothesis, it has been shown that DFP can inhibit the activity of FI in complex with C3b; however, no inhibition could be observed when FI was incubated with DFP in the absence of C3b (Ekdahl et al., 1990). This indicates that FI is able to weakly interact with C3b even in the absence of cofactors and that this binding induces a conformational change in FI. Direct, albeit low affinity binding (0.7 mM at physiological ionic strength) between FI and C3b was demonstrated to be ionic strength dependent (DiScipio, 1992). In the presence of FH, the affinity between C3b and FI increases many fold. Furthermore, it has been suggested that C3b undergoes a conformational change upon binding to FH that results in the accessibility of peptide bonds in C3b that can be cleaved by FI (Isenman, 1983). X-ray crystallography of the complex between C3b and complement control protein (CCP) domains 1–4 of FH showed that the first scissile bond in C3b is
exposed and likely accessible to FI. Cleavage of the other bond(s) subsequently requires conformational change (Janssen et al., 2006; Wiesmann et al., 2006; Wu et al., 2009). Intact SP domain was generated by proteolysis, and it was shown to have the same low activity toward small substrates as intact FI (Tsiftsoglou et al., 2005). The isolated SP is also unable to cleave C3b at a high level in a co-factor dependent fashion, instead it has a low level of cofactor independent activity cleaving C3b in a non-native pattern at more than the two usual sites. This suggests that the heavy chain plays a key role in orienting FI (along with the co-factor) but the absence of cleavage of C3b by intact FI may also hint an inhibitory activity of the heavy chain on the light chain SP.

Interestingly, it was observed that the FIMAC domains of C6/C7 bind the C345C domain of C5 (Thai and Ogata, 2004), which is highly homologous to C3, so it could be that the FI FIMAC binds the C345C of C3b/C4b. It is tempting to hypothesize that the FIMAC domain partially blocks the active site located in the SP domain or that it packs against the rest of the protein to maintain the SP in an inactive conformation.

Binding of the FIMAC domain to protein substrates then induces a conformational change in FI that allows proteolytic activity toward C3b/C4b presented in a proper conformation by the cofactors.

5. FI deficiency

The first case of FI deficiency was described in 1971 (Abramson et al., 1971) and since then over 30 families with complete FI deficiency have been reported; reviewed in (Nilsson et al., 2009). Patients presented with recurrent infections with encapsulated microorganisms (e.g. *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*). These symptoms are similar to patients deficient in C3 and are an illustration of the importance of C3 in opsonising microorganisms, enhancing ingestion by phagocytic cells. Interestingly, a few patients with complete FI deficiency have been reported to have renal disease or an autoimmune disorder (Amadei et al., 2001; Nilsson et al., 2009; Sadallah et al., 1999). It is difficult to say if these patients with complete FI deficiency develop glomerulonephritis and systemic lupus erythematosus due to lack of the FI protein and that they do not have the same inhibition of the complement system e.g. in the kidneys as healthy individuals or that missing FI predisposes to infections, which in turn promote development of autoimmune diseases.

Furthermore, a patient with a homozygous mutation in FI, which resulted in a subtotal deficiency, presented at the age of 46 years her first episode of atypical haemolytic uraemic syndrome (aHUS), an uncommon form of thrombotic microangiopathy with a poor renal prognosis (Bienaime et al., 2010). This and several other studies emphasized the role of FI in the pathogenesis of aHUS characterized by thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure.

The three first cases of heterozygous FI mutation with aHUS were reported in 2004 (Fremeaux-Bacchi et al., 2004). The clinical history of the three patients reported in this article are representative of the diversity of the clinical presentation of patients harbouring FI-associated aHUS. The first case is of a 32-year-old woman who presented after pregnancy with acute renal failure, hypertension and haemolytic anaemia with a rapid onset. A renal biopsy showed a thrombotic microangiopathy predominantly in glomeruli, with few vascular lesions, indicative for aHUS. The disease recurred two and four months later. One year later chronic haemodialysis was started. The second case is of a 17 month-old boy, who presented with aHUS characterised by severe microangiopathic haemolytic anaemia and proteinuria in the acute phase. A recurrence occurred six months later. Renal function remained stable between episodes of aHUS. No events were noted at the last follow up eight years later. The third case is a 26-year-old woman, referred to the renal unit at the time of an end-stage renal failure as result of aHUS. She received two kidney transplants complicated by acute rejection episodes with features of aHUS.

The frequencies of *CFI* mutations from five large aHUS cohorts (US, Italy, German, Spain and France) have been published during the last five years. In the published studies the *CFI* mutation frequency varies between 2% in the Spanish (n=41) and German cohorts (n=187) and 11.3% in the French cohort (n=202) (Le Quintrec et al., 2010; Maga et al., 2010).

To date 40 case reports with *CFI* mutations related to clinical descriptions have been published (Bienaime et al., 2010; Chan et al., 2009; Esparza-Gordillo et al., 2006; Geelen et al., 2007; Kavanagh et al., 2005; Kavanagh et al., 2008; Nilsson et al., 2007). The Italian and the French groups described the clinical outcome of a series of 12 and 23 FI-associated aHUS patients respectively (Noris et al., 2010; Sellier-Leclerc et al.,

2007). These data have been used in this review in order to describe the clinical features of FI-related aHUS genotype. The disease occurred below the age of four years or after 22 years. Of note, half of the patients presented with aHUS in adulthood with a maximal age at diagnosis of 55. In published Italian and French series 60% of FI-associated aHUS had a disease onset after the age of 17. The occurrence of an infectious triggering event is frequently noted in children. In the French paediatric cohort, aHUS onset following an infectious episode (upper respiratory tract infection, diarrhoea) was found in 63% of patients from all subgroups (Sellier-Leclerc et al., 2007). In adults, three of the published cases in females with *CFI* mutations are clearly linked to pregnancy (Fakhouri et al., 2010b). Extrarenal involvement during aHUS episodes (central nervous system ischemic manifestations, multivisceral manifestations of thrombotic microangiopathy) is not frequent (less than 10% in paediatric onset aHUS) (Sellier-Leclerc et al., 2007).

Gender ratio is equal in FI-associated aHUS of paediatric onset but more than 70% of the patients with adult onset are women. Familial FI-associated aHUS cases are rare. Among the 82 familial aHUS cases from the Italian registry, only 5% carried a *CFI* genetic defect, while 45% presented with a *CFH* genetic abnormality.

In eight reported familial aHUS two patients (two cousins; father and daughter; mother and son; sister and brother; two brothers) presented the disease at variable age of onset. Penetrance of aHUS is similar in the groups with *CFH*, *MCP* and *CFI* mutations and has been estimated to be approximately 50%, i.e. only half of the family members who carry the mutation develop the disease. It has been suggested that particular polymorphisms of *CFH* and *MCP* influence the predisposition to aHUS and may provide an explanation for the incomplete penetrance of the disease within families. In some families, it appeared that the proband had inherited the *CFI* mutation from one parent, and an allele carrying the polymorphism of *CFH* and/or *MCP* from the other parent, while the healthy mutation carriers did not inherit the aHUS-associated *CFH* and *MCP* polymorphisms (Esparza-Gordillo et al., 2006). In the French cohort, 35% of the FI-associated aHUS patients carried at least one additional known genetic risk factor for aHUS such as a mutation or a rare polymorphism in the *MCP*, *CFH*, *C3* or *CFB* genes. Another polymorphic variant of *CFH*-related genes (deletion of *CFHR1*) has been shown to be more frequent in FI-associated aHUS patients than in controls (Dragon-Durey et al., 2009).

The overall prognosis of FI-associated aHUS in the 40 reported cases is poor. More than 50% of published cases with *CFI* mutations developed end-stage renal disease or died during the first episode or within one year after the onset. Approximately the same frequency is observed in the FI-aHUS patients in the Italian registry (Noris et al., 2010) and in a French published series of 23 FI-associated aHUS patients (Bienaime et al., 2010). However in this study 30% of patients presented with complete remission of the disease. Apart from early recurrences within the first year after the onset, patients did not present any new aHUS episodes during extended follow-up (on average six years). Relapses with complete recovery have been observed only in one patient that presented with additional mutation in the *MCP* gene. Altogether these observations suggest that relapses remain rare in aHUS associated with *CFI* mutations.

Despite the small size of the French cohort, the analysis of the influence of the genotype on the outcome of the FI-associated aHUS patients provides evidence for the influence of the complement genetic background. Indeed the common polymorphism of the *CFHR1* gene (or a gene in linkage disequilibrium with the *CFHR1*) had a dramatic impact on the severity of the disease. Patients with a complete deletion of the *CFHR1* gene have significantly a poorer prognosis compared to patients with *CFI* mutation as a

unique susceptibility factor. Analysis of the clinical history of the French patients suggests that aHUS associated to *CFI* mutations as the sole risk factor has a good prognosis if there are no severe sequelae after the first episode. These results strongly suggest that the complement genetic background influences the severity of aHUS disease and emphasize the necessity of genetic screening for all susceptibility factors in patients with aHUS in order to establish an "identity card" of individual aHUS risk factors and to propose tailored therapeutic approaches.

Altogether, it therefore appears that *CFH*-mutated patients are at the highest risk of ESRD very early during the course of the disease, while *CFI*-mutated patients progress to ESRD mainly during the first year after the onset. Renal transplantation in aHUS patients with *CFI* mutations has been associated with a high recurrence rate and poor prognosis (review in (Zuber et al., 2011)). The reported cases comprise 15 renal transplants, received by ten patients. Twelve renal grafts were lost due to a HUS recurrence (80%).

Heterozygous mutations in FI are also associated with forms of C3 glomerulopathy (Fakhouri et al., 2010a). A peculiar primary glomerulonephritis characterized by isolated mesangial C3 deposits in the absence of mesangial proliferation was reported on the background of heterozygous mutations in the *CFI* genes (Servais et al., 2007). Recently two heterozygous mutations in the *CFI* gene were reported in a context of immune-complex-mediated membranoproliferative glomerulonephritis type I, which is an additional illustration of the link between *CFI* mutations and C3 glomerulopathy (Leroy et al., 2010; Leroy et al., 2011). One of these mutations has been previously reported in a patient with aHUS, and has been associated with a quantitative FI deficiency. The functional consequences and the potential disease-relevance of the last two mutations are not known and the relevance of these mutations in kidney disease development remains speculative.

The mechanism by which complement dysregulation leads to aHUS is not fully elucidated yet. However, it appears that deficiencies in control mechanisms such as impaired FI lead to generation of anaphylatoxins and MAC that activate the endothelium leading to a pro-coagulant phenotype (Tedesco et al., 1997). Activated endothelium expresses in turn tissue factor that initiates coagulation cascade (Saadi et al., 1995). Platelets can be activated by anaphylatoxins and thereafter attach more easily to activated glomerular endothelium (Polley and Nachman, 1983) propagating further coagulation and thrombus formation in the glomeruli.

Purified or recombinant FI is not yet available for treatment of FI deficient patients but may be a good therapeutic option particularly in the patients with partial FI deficiency that have low risk of developing autoantibodies to FI.

6. Conclusions

FI is an unusual protease as it has no inhibitor and its activity is regulated by a narrow specificity towards two substrates, C3b and C4b, which are only cleaved in the presence of cofactors. The 3D structures of FI alone and in complex with its substrates and cofactors are long awaited particularly in the view of severe diseases affecting patients lacking FI.

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Figure legends

Figure 1. Schematic structure of *CFI* gene and resulting protein. The glycosylations are marked with arrows.

Figure 2. Impaired complement regulation in aHUS due to mutations in complement inhibitors including FI. In order to protect the endothelial cells in the glomerulus from injury, complement activation is inhibited by membrane-bound (MCP) and soluble inhibitors (FI and FH) (Kajander et al., 2011; Morgan et al., 2011). C3b bound to its cofactors such as FH and MCP is cleaved to iC3b and C3f fragments. When FI is dysfunctional due to mutations, the complement cascade proceeds further via the alternative pathway leading to generation of the anaphylatoxins C3a and C5 as well as MAC. The binding of C5a to C5a-receptor stimulates the endothelial cells to express P-selectin and von Willebrand factor (vWF) (Foreman et al., 1994). Also MAC induces expression of P-selectin (Bossi et al., 2008). The activated neutrophils bind to P-selectin and release reactive oxygen species (ROS) and proteinases, which further damage the endothelial cells. The basement membrane becomes exposed, vWF and tissue factor (TF) are released and this attract the platelets to adhere and aggregate to the exposed matrix. These platelets together with fibrinogen form a thrombus (Goldberg et al., 2010).

Table 1. Outcome of FI-associated aHUS patients reported in the Italian and French cohorts.

References	Cohort	No. of patients	Child/Adult	Low level C3	Low level FI	Outcome after the first aHUS flare			Long term outcome > 1 year	
						ESRF	Death	Recurrence	ESRF	Death
(Noris et al., 2010)	Italy	10	4/6	2/10	nd	6/10	0/10	nd	6/10	0/10
(Bienaime et al., 2010)*	France	15	4/11	5/15	6/14	5/15	1/15	2/15	8/15	1/15
Total (%)		25	47%	28%	42%	44%	4%	10%	56%	4%

*Patients without additional identified mutations. ESRF = end-stage renal failure. Nd – not determined.

Table 2. Mutations found in *CFI* in complete and partial FI deficiency.

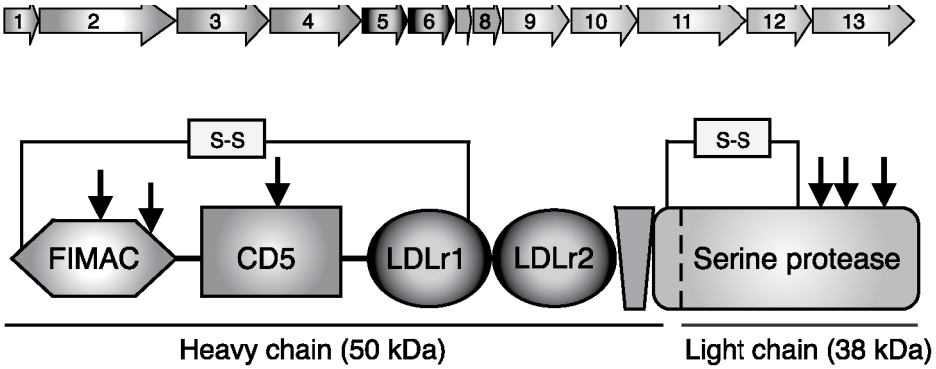
Mutations found in CFI in complete FI deficiency

Genetic status	DNA ATG +1	Protein without signal peptide	Exon	Domain	Function	Symptoms	References
Homo	c.133-134delAA	p.K27SfsX11	2	FIMAC	Lack of secretion	Recurrent infections with *S. pneumoniae* and *S. pyogenes Septicaemia*, joint infections and pneumonia. Juvenile idiopathic arthritis.	(Nita et al., 2011)
Hetero	c.563G>T	G170V	4	CD5	Lack of secretion	Recurrent upper respiratory infections. Two severe bacterial infections (sepsis and erysipelas).	(Nilsson et al., 2009)
Hetero	c.748C>A	Q232K	5	LDLr1	Impaired secretion	No infections, SLE	(Nilsson et al., 2009)
Homo	c.764G>A	C237Y	5	LDLr1	Lack of secretion	Recurrent upper respiratory tract infections. Vasculitis.	(Genel et al., 2005; Nilsson et al., 2009)
Hetero	c.772G>A	A240T	5	LDLr2	Interrupting a exon/ intron boundary, mRNA splicing	Recurrent upper respiratory tract infections. Pneumonia. Arthralgia.	(Nilsson et al., 2009; Vyse et al., 1996)
Hetero	c.803C>T	S250L	6	LDLr2	Impaired secretion	No infections, SLE	(Nilsson et al., 2009)
Homo	c.866A>T	D271V	6	LDLr2	Lack of secretion	Recurrent upper and lower respiratory system infections. Vasculitis and	(Nita et al., 2011)

						arthralgia.	
Homo	c.1060C>T	Q336X	10	SP	Truncated protein degraded	Recurrent upper respiratory tract infections. Pneumonia. Recurrent episodes of purpuric rash in the lower extremities.	(Nilsson et al., 2009)
Hetero	c.1071T>G	I339M [a]	10	SP	Lack of secretion	Recurrent upper respiratory tract infections. Pneumonia. Arthralgia.	(Nilsson et al., 2009)
Homo	c.1174insAT	p.I374IfsX5	11	SP	Lack of secretion	Infections, SLE, glomerulonephritis	(Baracho et al., 2003)
Hetero	c.1253A>T	H400L	11	SP	Lack of secretion	Recurrent upper respiratory infections. Two severe bacterial infections (sepsis and erysipelas).	(Nilsson et al., 2009; Vyse et al., 1996)
Mutations found in CFI in partial FI deficiency							
Hetero	c.128G>T	C25F	2	FIMAC	Lack of secretion	aHUS	(Bienaime et al., 2010)
Hetero	c.148C>G	P32A	2	FIMAC	Impaired function on the surface	aHUS	(Bienaime et al., 2010)
Hetero	c.353A>G	H100R	3	CD5	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.355G>A	G101R	3	CD5	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.412A>G	M120V	3	CD5	Normal	aHUS	(Nilsson et al., 2010)
Hetero	c.414G>T	M120I	3	CD5	Normal	aHUS	(Bienaime et al., 2010; Kavanagh et al., 2008)
Hetero	c.434G>A	W127x	3	CD5	Lack of secretion	aHUS	(Kavanagh et al., 2005)
Hetero	c.452A>G	N133S	3	CD5	Lack of secretion	aHUS	(Bienaime et al., 2010; Nilsson et al., 2010)
Hetero	c.454G>A	V134M	3	CD5	nd	aHUS	(Westra et al., 2010)
Hetero	c.548A>G	H165R	4	CD5	Normal	aHUS	(Boyer et al., 2008; Nilsson et al., 2010)
Hetero	c.719C>G	A222G	5	LDLr1	Impaired function on the surface	aHUS	(Caprioli et al., 2006; Nilsson et al., 2010)
Hetero	c.739T>G	C229G	5	LDLr1	nd	aHUS	(Cruzado et al., 2009)
Hetero	c.782G>A	G243D	6	LDLr2	Normal	aHUS	(Kavanagh et al., 2008; Nilsson et al., 2007)
Hetero	c.784delA	p.G243fsX46	6	LDLr2	Lack of secretion	aHUS	(Bienaime et al., 2010)
Hetero	c.917T>G	I288S	8	Unknown region	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.893delC	L289x	8	Unknown region	Lack of secretion	aHUS	(Kavanagh et al., 2005)
Hetero	c.949C>T	R299W	9	SP link	Reduced expression	aHUS	(Caprioli et al., 2006; Kavanagh et al., 2008)
Hetero	c.1009C>T	I322T	9	SP link	nd	aHUS	(Geelen et al., 2007; Kavanagh et al., 2008; Westra et al., 2010)

Hetero	c.1106A>C	Y351S	10	SP	nd	aHUS	(Chan et al., 2009)
Hetero	c.1207G>A	D385N	11	SP	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.1216C>T	R388C	11	SP	nd	aHUS	(Moore et al., 2010)
Hetero	c.1246A>C	I398L	11	SP	Lack of secretion	aHUS	(Bienaime et al., 2010; Sellier-Leclerc et al., 2007)
Hetero	c.1271G>A	G406D	11	SP	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.1291G>A	A413T	11	SP	Lack of secretion	aHUS	(Bienaime et al., 2010)
Hetero	c.1297A>G	I415V	11	SP	Impaired function on the surface	aHUS	(Sellier-Leclerc et al., 2007)
Hetero	c.1367G>T	W438L	11	SP	Lack of secretion	aHUS	(Bienaime et al., 2010)
Hetero	c.1376A>C	Y441S	11	SP	nd	aHUS	(Bienaime et al., 2010)
Hetero	c.1420C>T	R456x	11	SP	Lack of secretion	aHUS	(Bienaime et al., 2010; Fremeaux-Bacchi et al., 2004; Nilsson et al., 2010; Westra et al., 2010)
Hetero	c.1446-1450 del TTCAC	L466V, Q467G, W468x	12	SP	Lack of secretion	aHUS	(Caprioli et al., 2006; Nilsson et al., 2010)
Hetero	g.IVS12+5g>t [b]		Intron 12		Splice score decrease from 93 to 86	aHUS	(Caprioli et al., 2006; Westra et al., 2010)
Hetero	c.1555G>A	D501N	13	SP	Abolished function on the surface	aHUS	(Caprioli et al., 2006; Nilsson et al., 2010)
Hetero	c.1571A>T	D506V	13	SP	Slightly impaired function on the surface	aHUS	(Bienaime et al., 2010; Fremeaux-Bacchi et al., 2004; Kavanagh et al., 2008)
Hetero	c.1610insAT	D519V, T520x	13	SP	Lack of secretion	aHUS	(Esparza-Gordillo et al., 2006; Nilsson et al., 2010)
Hetero	c.1637G>A	W528x	13	SP	Lack of secretion	aHUS	(Fremeaux-Bacchi et al., 2004; Nilsson et al., 2010)
Hetero	c.1657C>T	P535S	13	SP	nd	aHUS	(Bienaime et al., 2010)

nd – not determined, [a] This mutation was also identified in a patient with aHUS (Westra et al., 2010); [b] appears to be rare variant, has been identified in 5 of 183 healthy French individuals (1.3%) and in 8 of the 238 patients from the French multi center aHUS cohort (1.7 %) (VFB personal communication).


1
2
3
4
5
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8
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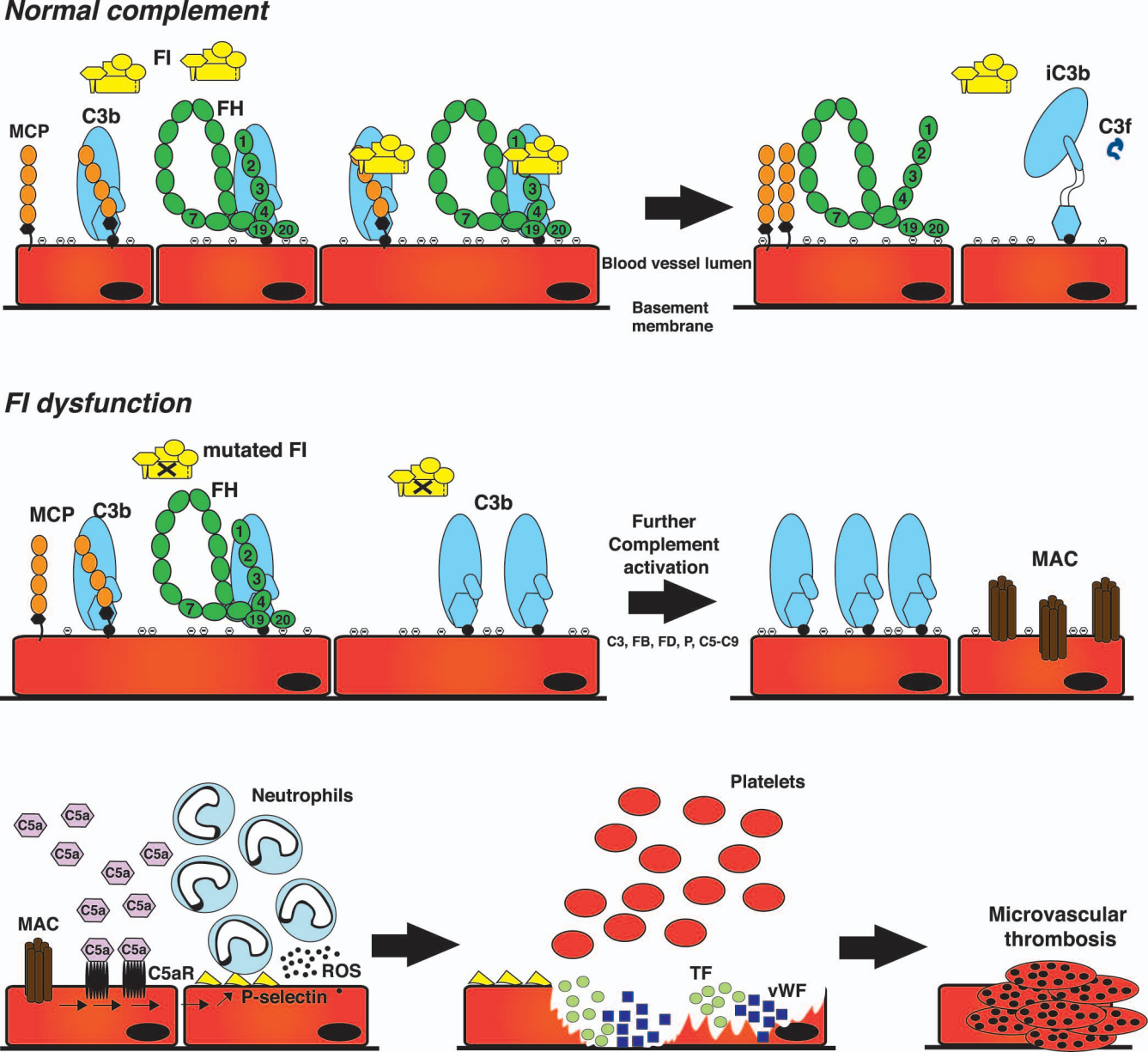
10
11
12
13
S-S
S-S
FIMAC
CD5
LDLr1
LDLr2
Serine protease
Heavy chain (50 kDa)
Light chain (38 kDa)


Normal complement
FI
C3b
FH
MCP
1
2
3
4
7
19
20
iC3b
C3f
Blood vessel lumen
Basement membrane
FI dysfunction
mutated FI
FH
MCP
C3b
C3b
Further Complement activation
C3, FB, FD, P, C5-C9
MAC
Neutrophils
C5a
MAC
C5aR
ROS
P-selectin
Platelets
TF
vWF
Microvascular thrombosis