Review

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Factor H-related proteins determine complement-activating surfaces

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Complement factor H-related proteins (FHRs) are strongly associated with different diseases involving complement dysregulation, which suggests a major role for these proteins regulating complement activation. Because FHRs are evolutionarily and structurally related to complement inhibitor factor H (FH), the initial assumption was that the FHRs are also negative complement regulators. Whereas weak complement inhibiting activities were originally reported for these molecules, recent developments indicate that FHRs may enhance complement activation, with important implications for the role of these proteins in health and disease. We review these findings here, and propose that FHRs represent a complex set of surface recognition molecules that, by competing with FH, provide improved discrimination of self and non-self surfaces and play a central role in determining appropriate activation of the complement pathway.

Introduction

The complement system is an essential part of innate immunity, with decisive roles in protection against infection but also participating in the modulation of inflammation, the disposal of immune complexes, cellular waste, and apoptotic/necrotic cells, and the activation and regulation of innate and adaptive immune cells [1]. Preserving the physiological balance between complement activation and inhibition is critical to maintaining homeostasis and involves targeting appropriate sites for activation while simultaneously avoiding bystander damage to host tissues. This is ensured by target recognition mechanisms that initiate and amplify complement activation only when necessary, through the built-in specificity and limited activity of the enzymatic complexes (C3/C5 convertases) of the system and by an array of receptors and regulatory molecules that control the activation cascade at different steps. Imbalance between activation and inhibition due to excessive activation or improper regulation has pathological consequences [2–5].

FH is the main regulator of the alternative pathway of complement, both in fluid phase and on cellular surfaces. FH is a relatively abundant plasma protein that is essential in restricting the action of complement to activating surfaces. FH binds to C3b, accelerates the decay of the alternative pathway (AP) C3 convertase (C3bBb) and acts as a cofactor for the factor I (FI)-mediated proteolytic degradation of C3b (Box 1). Recent data indicate that the complement regulatory activities of FH (see Glossary) on self and non-self surfaces are modulated by a group of

Glossary

Age-related macular degeneration (AMD): a major cause of blindness among the elderly with multiple predisposing factors including complement gene variants; characterized by the accumulation of waste material along BM in the retina.
Atypical hemolytic uremic syndrome (aHUS): a form of thrombotic microangiopathy characterized by hemolytic anemia, low platelet count, and acute renal failure, with predisposing complement gene variants or autoantibodies to FH.
C3 glomerulopathy (C3G): various forms of glomerulonephritis characterized by involvement of the alternative complement pathway and C3 deposition in the glomeruli with absent or scanty immunoglobulin accumulation.
Cofactor activity: the ability of FH and some other complement regulators to assist the serine protease FI in the enzymatic degradation of the central complement fragment C3b.
Complement deregulation: the ability of the FHR proteins to competitively inhibit the complement regulator FH.
Decay-accelerating activity: the capacity of FH and a few other complement regulators [decay-accelerating factor (DAF), complement receptor type 1 (CR1)] to facilitate the disassembly of the C3 convertase enzyme C3bBb.
Haplotype: a set of DNA variations (i.e., mutations, SNPs, copy number variations) found on the same chromosome and that tend to be inherited together.
IgA nephropathy (IgAN): a form of glomerulonephritis characterized by IgA-containing immune complexes.
Malondialdehyde (MDA) epitopes: epitopes that originate from peroxidation of membrane lipids due to oxidative stress and that modify primary amino groups in proteins and lipids.
Membrane attack complex (MAC): a pore formed by complement proteins C5b, C6, C7, C8, and C9 in the cell membrane of target cells.
Short consensus repeat (SCR) or Sushi domain: also known as the complement control protein (CCP) domain, a domain characteristic of many complement regulatory and other proteins.
Systemic lupus erythematosus (SLE): a systemic autoimmune disease characterized by anti-DNA and other autoantibodies.

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Box 1. Complement activation and its regulation by FH

Pathogens, altered host cells or molecules, and certain host ligands released/exposed during infections and inflammatory processes, such as pentraxins, molecules of the ECM, and DNA, can activate complement. The classical complement pathway is activated by the binding of C1q to immunoglobulins or pentraxins or by the direct binding of C1q to such ligands/surfaces. The lectin pathway is initiated via target-bound mannose-binding lectin or ficolins. The C3b fragment generated from C3 through these activation routes feeds into the AP, amplifying the classical and lectin pathway-mediated activation. The tick-over mechanism [80] ensures low-rate, constant activation of C3 in plasma through the AP. Properdin may also act as an AP initiator [81]. Cleavage of C3 into C3b results in the covalent binding of C3b to the activating surface. Incorporation of additional C3b molecules to the surface-bound AP C3 convertases generates the C5 convertases with the capacity to bind and cleave C5, leading to the initiation of the lytic pathway and the generation of terminal complement complexes (termed MAC when integrated into target cell membranes) (Figure I). Regulation of complement activation is a necessary and complex process involving several soluble and membrane-associated proteins, including C4b-binding protein (C4bp), FH, membrane cofactor protein (MCP) (CD46), CR1 (CD35), and DAF (CD55) (Figure I). These proteins are essential to control the C3/C5 convertases by either catalyzing the proteolytic degradation of C3b/C4b by FI (MCP, CR1, FH, C4 bp) or accelerating their dissociation (DAF, CR1, FH, C4 bp). Among them, FH is the prototypical member and the main regulator of the AP. FH regulates complement both in fluid phase and on cellular surfaces. However, while FH binds and inactivates promptly C3b in fluid phase, the inactivation of surface-bound C3b by FH is dependent on the chemical composition of the surface to which C3b is bound. In the presence of polyanions like sialic acids, glycosaminoglycans, or sulfated polysaccharides (heparins), the affinity of FH for surface-bound C3b increases as a consequence of the simultaneous recognition of both polyanionic molecules and bound C3b by the same FH molecule [58,69,82,83].

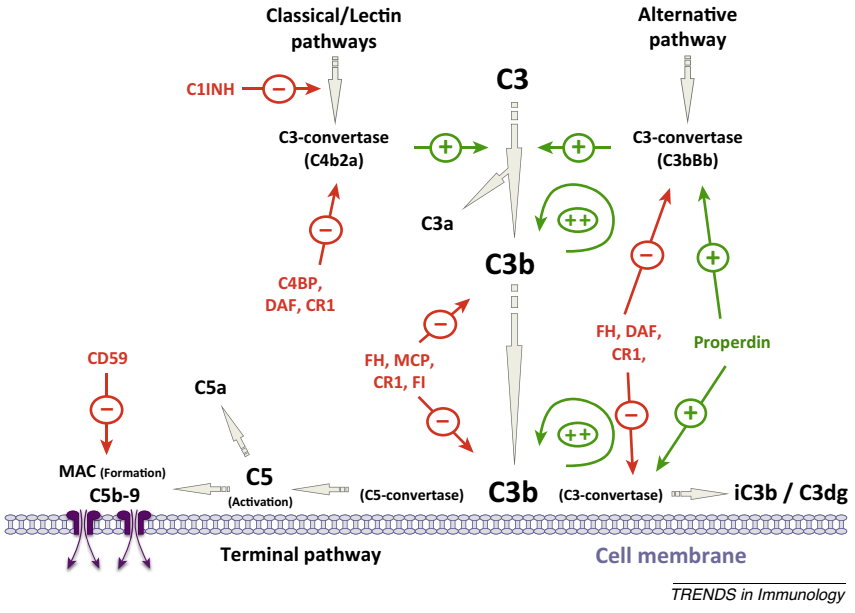

Figure I. Complement activation by the three activation pathways results in the formation of C3 convertases that cleave the C3 molecule, generating C3b, which may organize additional alternative pathway (AP) C3 convertase molecules amplifying complement activation on fluid phase and surfaces (circle arrows). C3 activation on surfaces results in formation of C3/C5 convertase, which cleaves C5 and triggers inflammation and cell lysis through the terminal pathway. Our simplified view of the complement activation cascade also illustrates that regulation of the AP amplification loop and surface deposition of C3b are complex processes involving several soluble and membrane-associated proteins (depicted in red). The activity of these proteins, accelerating the dissociation of the C3/C5 convertases or degrading the C3b molecule into iC3b and C3dg, preserves complement homeostasis and limits the action of complement to the activating surfaces. Abbreviations for complement proteins depicted in the figure are: C1INH, C1 inhibitor; C4 bp, C4b-binding protein; MCP, membrane cofactor protein (CD46); DAF, decay-accelerating factor (CD55); FH, factor H; and CR1, complement receptor type 1 (CD35).

evolutionarily and structurally related proteins termed FHR proteins. Here we review these recent developments, place them within the context of the current understanding of mechanisms regulating the complement pathway, and discuss the implications that this new understanding has in health and disease.

The FH/FHR protein family

The FH/FHR protein family is encoded by six genes positioned in tandem. This family includes FH and FHL-1, both derived from *CFH* via alternative splicing, and the FHR-1, -2, -3, -4, and -5 proteins encoded by the *CFHR1–5* genes. FHR-1 has two allelic variants, FHR-1A and FHR-1B, and there are two isoforms of FHR-4, FHR-4A and FHR-4B (Figure 1). All of these proteins comprise repetitive units of approximately 60 amino acids named short consensus repeats (SCRs), arranged in a continuous fashion. The *CFHR1–5* genes originated from the *CFH* gene by tandem duplication events [6] (Figure 1A). The C-terminal region (SCRs 18–20) and SCRs 6/7 of FH, harboring the major surface recognition sites of FH, are retained with differing degrees of conservation in all FHRs, explaining their capacity to interact with most of the FH ligands (Table 1) [7–9]. However, none of the FHRs contains regions homologous to FH SCRs 1–4, which has called into question the conservation of the complement regulatory activities of FH in the FHRs (Figure 1B,C).

A remarkable characteristic of FHR-1, FHR-2, and FHR-5 is that the two N-terminal SCRs, which are almost identical in these FHRs (Figure 1C), include a dimerization

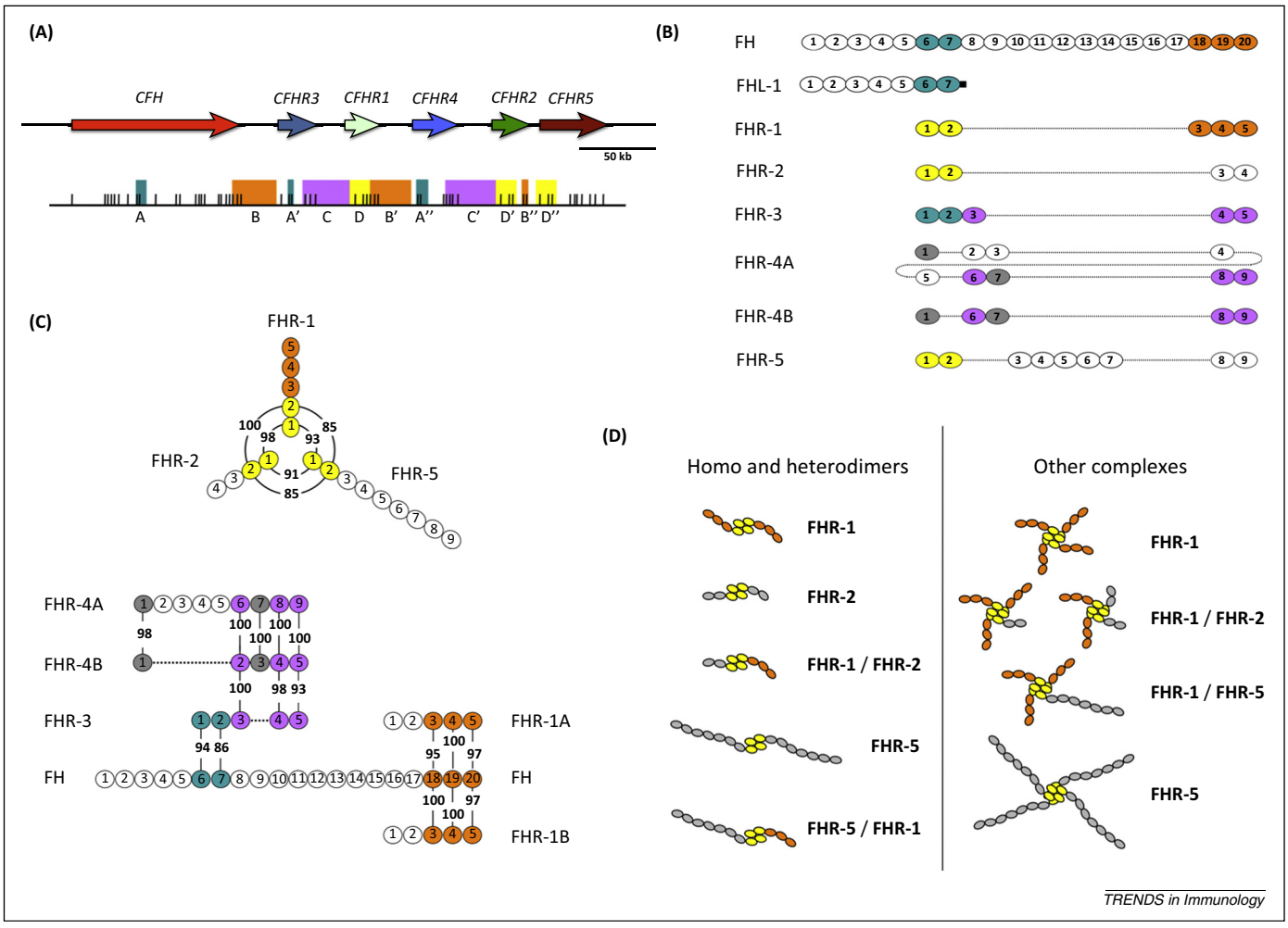

Figure 1. The factor H (FH)/factor H-related (FHR) family of proteins. **(A)** Genomic organization of the *CFH* and *CFHR1–5* genes. Arrows represent the genes with their names. The colored boxes underneath indicate the sequence repeats. The vertical lines indicate the position of the exons of the *CFH* and *CFHR* genes. **(B)** Structural organization of the FH and FHR proteins. Short consensus repeats (SCRs) are represented by ovals and are numbered from the N-terminal end. Homologous SCRs are aligned. Colors illustrate SCRs exhibiting almost complete identity of their amino acid sequences. **(C)** Percentages of amino acid similarities are provided for SCRs 1 and 2 between FHR-1, FHR-2, and FHR-5, SCRs 6 and 7 in FH, and SCRs 1 and 2 in FHR-3. The diagram also includes alignments to illustrate the similarities between the C-terminal regions of FH and the two FHR-1 alleles, as well as between FHR-3 and the two FHR-4 isoforms. **(D)** FHR-1, FHR-2, and FHR-5 complexes for which there is experimental evidence. Models are drawn based on structural data demonstrating that the first two N-terminal SCRs of these proteins form dimers in a head-to-tail orientation [10]. For simplicity, tetramers are also depicted showing interactions through these SCRs.

domain [10]. This determines that these FHRs, in contrast to FH, always circulate in plasma as dimers or tetramers (Figure 1D) [10,11]. This structural organization has major functional implications. Homo- and hetero-oligomerization increased the avidity of FHR-1, FHR-2, and FHR-5 for their ligands C3b, iC3b, and C3dg and polyanions like sialic acids, glycosaminoglycans, and heparin. In addition, because the FHR composition of the different oligomers influences the carbohydrate-binding characteristics for each of these FHR complexes, a combinatorial repertoire of different FHRs is likely to provide finer identification of opsonized surfaces with differing carbohydrate compositions and differing densities and rates of deposition of C3 fragments.

FHRs and disease

Genetic variations in the FHRs are associated with various diseases including C3 glomerulopathy (C3G), atypical hemolytic uremic syndrome (aHUS), IgA nephropathy (IgAN), systemic lupus erythematosus (SLE), and age-related macular degeneration (AMD) [12–24]. Among them, variations involving *CFHR1–5* genomic rearrangements (Table 2) are the most remarkable and informative. These rearrangements are not unusual because the region contains large genomic duplications (Figure 1A), which makes it highly prone to genomic rearrangements through gene conversion and non-allelic homologous recombination [6].

Rearrangements resulting in the generation of hybrid genes between *CFH* and *CFHR1* or *CFHR3* strongly associate with aHUS, a rare kidney disease characterized by impaired regulation of complement activation on endothelial surfaces leading to thrombotic microangiopathy. Notably, FH proteins in which the last C-terminal SCR20 has been replaced by the C-terminal SCR5 of FHR-1 or by the whole FHR-3 – namely, FH::FHR-1 and FH::FHR-3 hybrids – are unable to regulate complement properly on endothelial surfaces [25–28]. Similarly, the FHR-1::FH hybrid protein in which the C-terminal SCR5 of FHR-1 has been replaced by SCR20 of FH also impairs

Table 1. Known host ligands of the human FHR proteins

Protein	Ligand	Associated function	Refs
FHR-1	C3b, C3dg, iC3b	Competition with FH (no cofactor/decay-accelerating activity)[a]	[10,11,39,45]
	Heparin	Surface recognition?	[45]
	PTX3	Surface recognition?	[69]
	C5	Terminal pathway inhibition (questioned by others)[a]	[10,45,84]
	Lipoproteins (HDL)	Unknown	[85]
	CR3	Cellular adhesion	[56]
FHR-2	C3b, C3dg, iC3b	Inhibition of C3 convertase (no cofactor or decay-accelerating activity), terminal pathway inhibition[a]	[44]
	Heparin	Surface recognition?	[44]
	Lipoproteins (HDL)	Unknown	[85]
FHR-3	C3b, C3dg, iC3b	Enhancement of FH cofactor activity	[40]
	C3b	Cofactor activity[a]	[42]
	Heparin	Surface recognition?	[40]
FHR-4A, FHR-4B	C3b, C3dg, iC3b	Enhancement of FH cofactor activity	[40,55]
	C3b	Enhancement of AP activation	[55]
	CRP	Enhancement of CP activation	[51]
	Lipoproteins (chylomicrons, LDL, VLDL)	Unknown	[86]
FHR-5	C3b, C3dg, iC3b	Inhibition of C3 convertase; weak cofactor activity[a], competition with FH	[10,41]
	PTX3	Competition with FH	[68]
	Heparin	Surface recognition?	[41]
	CRP	Surface recognition? Competition with FH	[41,68]
	Lipoproteins (HDL)	Unknown	[41]

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

[a]The physiological nature of these activities is discussed in the text.

complement regulation by competing with binding of FH to the endothelium [29]. As a whole, data generated from studies of the *CFH::CFHR1/CFHR3* hybrid genes [25–27,29–31] illustrate the critical role that the C-terminal region of FH plays in the protection of host surfaces from complement damage and that this region in FH cannot be replaced by the C-terminal region of FHR-1 or FHR-3; FH and FHR-1/FHR-3 have different binding specificities. Notably, the observation that the FHR-1::FH hybrid protein impairs complement regulation also indicates that FHR-1 lacks appropriate complement regulatory activity to substitute FH [29].

A second type of rearrangement in the *CFH/CFHR1–5* region is the deletion of the *CFHR3* and *CFHR1* genes. This is a common polymorphism in humans, with allelic frequencies ranging from 0 to 0.55 in different populations [32], that originated from a single non-allelic homologous recombination event involving a duplicated region downstream of *CFH* and *CFHR1* [33]. Remarkably, the *CFHR3–CFHR1* deletion is strongly associated with lower risk of AMD [17] and IgAN [16], two prevalent conditions affecting the retina and the kidney, respectively. By contrast, the *CFHR3–CFHR1* deletion is a risk factor for SLE, autoimmune aHUS, and hematopoietic stem cell transplantation-related thrombotic microangiopathy associated with the generation of anti-FH autoantibodies [24,32,34,35].

Finally, there is a set of genomic rearrangements resulting in the duplication of the dimerization domain of FHR-1, FHR-2, and FHR-5 that are specifically associated with C3G, a heterogeneous group of rare glomerulopathies leading to renal failure characterized by massive deposition of C3 derivatives along the glomerular basement membrane (GBM) [11,12,14,15,36,37]. This duplication of the dimerization domain causes abnormal multimerization of FHR-1, FHR-2, and FHR-5, which increases avidity for their ligands and enhances competition with FH [10,11]. It is postulated that these gain-of-function mutant FHR-1, FHR-2, and FHR-5 proteins are pathogenic because they over-compete with FH binding to host surfaces and impair complement regulation.

As described, the association of the *CFH/CFHR* rearrangements with disease suggests that FHRs originated to modulate complement activation by competing with the binding of FH to surfaces and that this competition can be beneficial or detrimental depending on the surfaces/circumstances.

The evolving understanding of the role of the FHR proteins

Because of their homology with FH, complement regulatory roles were originally postulated for the FHRs [38]. The initial studies, however, could not demonstrate FH-like activity for these proteins [39]. Subsequent studies showed weak cofactor activities of FHR-3, FHR-4, and FHR-5 [40,41] and synergistic activity with FH; namely, enhancement of the cofactor activity of FH in the case of FHR-3 and FHR-4 [40]. Notably, however, high and now known to be nonphysiological concentrations of the FHR-3 and FHR-4 proteins were required for these activities. FHR-5 was also reported to inhibit the alternative pathway (AP) C3 convertase [41] and, more recently, strong cofactor activity was shown for FHR-3 [42], although this again required a concentration above the likely (yet precisely undefined) plasma concentration of FHR-3 [43]. One study reported a complement inhibiting function for FHR-2 by inhibiting the activity of the C3 convertase (i.e., C3 cleavage) and by

Table 2. CFHR gene rearrangements associated with disease

Disease		Genetic finding	Outcome	Significance	Risk/ Protection	Prevalence	Refs
aHUS		*CFH::CFHR1* hybrid genes	Substitution of the C-terminal SCR of FH for that in FHR-1	Loss of complement regulation at cell surfaces	R	Several unrelated cases described	[28]
		CFHR1::CFH hybrid genes	Substitution of the C-terminal SCR of FHR1 for that in FH Two C-terminal SCRs for those of FH	Loss of complement regulation at cell surfaces	R	Few unrelated cases described	[29,87]
		CFH::CFHR3 hybrid gene	Substitution of the last C-terminal SCR20 of FH for the whole FHR-3	Loss of complement regulation at cell surfaces	R	Very rare	[88]
		DelCFHR3–CFHR1 DelCFHR1–CFHR4	Loss of FHR-3 and FHR-1 Loss of FHR-1 and FHR-4	Associated with FH autoantibodies impairing cell surface regulation	R	Common	[12,35,89,90]
C3G	Dense deposit disease	*DupCFHR1*	Mutant FHR-1 with SCR123412345	Abnormal oligomerization, increased competition with FH	R	Very rare	[11]
		CFHR2::CFHR5 hybrid gene	Hybrid protein containing SCR1/2 of FHR-2 followed by the whole FHR-5 molecule	Abnormal oligomerization, increased competition with FH	R	Very rare	[14]
	C3 glomerulonephritis	*CFHR3::CFHR1* hybrid gene	Hybrid protein containing SCR1/2 of FHR-3 followed by the whole FHR-1 molecule	Increased levels of FHR-1, increased competition with FH?	R	Very rare	[19]
		CFHR5::CFHR2 hybrid gene	Hybrid protein containing SCR1/2 of FHR-5 followed by the whole FHR-2 molecule	Increased levels of FHR-2 Increased competition with FH?	R	Very rare	[66]
		DupCFHR5	Mutant FHR-5 with SCR12123456789	Abnormal oligomerization, increased competition with FH	R	Several related cases described, one unrelated	[10,15,91]
AMD		*DelCFHR3–CFHR1*	Loss of FHR-3 and FHR-1	No competition with FH?	P	Common	[17]
IgAN		*DelCFHR3–CFHR1*	Loss of FHR-3 and FHR-1	No competition with FH?	P	Common	[16]
SLE		*DelCFHR3–CFHR1*	Loss of FHR-3 and FHR-1	No competition with FH?	R	Common	[24]

preventing assembly of the lytic terminal complement complex [44]. Another study showed that FHR-1, instead of having FH-like regulatory activity, inhibits the lytic pathway [45], although others demonstrated that FHR-1 could not significantly influence and inhibit complement-mediated lysis of sheep erythrocytes [29,46].

The ability of recombinant FHR-1 and FHR-5 proteins or native FHR-1, FHR-2, and FHR-5 oligomers to regulate C3b and the AP has now been more extensively analyzed using hemolytic assays and surface plasmon resonance. These analyses demonstrated that these FHRs bind to C3b, iC3b, and C3dg but provided no evidence of cofactor activity for the FI-mediated proteolysis of C3b or AP C3 convertase decay-accelerating activities [10,11]. These studies also failed to detect any significant interaction of FHR-1 with C5 [10], indicating that FHR-1, FHR-2, and FHR-5 have no intrinsic C3 or C5 regulatory activity at physiological concentrations. By contrast, these experiments showed that the FHR-1, -2, and -5 proteins, through their ability to compete with FH for binding to C3b, actually prevent FH-mediated complement regulation. This interference with the FH regulatory activities is apparently facilitated by their oligomerization, which increases avidity for their ligands, and is significantly enhanced by the rare C3G-associated mutations that result in abnormal multimerization of the FHR-1, FHR-2, and FHR-5 proteins [10,11]. Importantly, it has been reported that FHR-3 and FHR-4 are also able to compete for FH ligands and therefore have the potential to interfere with FH regulation [42,47].

In addition to playing important roles in controlling FH activities on self surfaces, FH and some of the FHRs have also been found to interact with several microbes and microbial proteins. However, only a few studies have addressed the functional consequences of the interaction of the FHR proteins with pathogens. These studies showed that none of the bound FHRs (FHR-1, FHR-2, and FHR-5) conferred protection from complement activation and deposition of C3b and C5b-9, whereas binding of FH could reduce or completely block C3b and C5b-9 deposition and lysis [48]. This suggests that FH but not the FHRs protect microbes from opsonophagocytosis and/or complement-mediated damage when bound on the microbial surface. Strikingly, the FH domains that are well conserved among the FHRs are those that mediate the binding of FH to most known FH-binding pathogens and microbial proteins (Figure 2), raising the possibility that the FHRs have evolved as decoys to reduce the amount of FH bound by the pathogens and, consequently, potentiate their elimination mediated by the host complement system.

Recent experiments with the major human pathogen *Neisseria meningitidis* provide strong support for these ideas [47]. *N. meningitidis* recruits FH via fHbp, a surface lipoprotein that mimics host carbohydrates and binds FH with high affinity [49]. In their report, Caesar *et al.* elegantly show that FHR-3 binds fHbp with similar affinities to FH and that FHR-3 competes with FH on the bacterial surface, influencing *N. meningitidis* survival in serum sensitivity assays. These findings may explain the earlier observation that individuals carrying a particular extended *CFH–CFHR3–CFHR1* haplotype exhibit significant protection against *N. meningitidis* infections [50]. Notably, this haplotype (*H3* haplotype), which is also an important risk factor for aHUS, determines significantly lower levels of FH and includes polymorphisms in potential *CFHR3* regulatory regions that may also affect FHR-3 expression in plasma (Bernabeu-Herrero *et al.*, submitted).

Some FHRs seem to have acquired functions that enhance complement activation on the surface of pathogens. For example, FHR-4 binds preferentially to the native, pentameric form of C-reactive protein (CRP) (pCRP) and can thus allow complement activation [51,52] whereas FH binds mainly the modified, monomeric form of CRP (mCRP) at lower concentrations [52–54]. Similarly, both FHR-4 and FH bind to C3b but only FH promotes inactivation of C3b efficiently; FHR-4 rather enhances complement activation by allowing AP convertase formation on FHR-4-bound C3b [55]. This mechanism may result in enhanced opsonization of dying cells or some pathogens in the presence of FHR-4 [52,56,57].

Taken together, all of these recent data suggest that the FHRs modulate complement activation by competing with FH for binding to its ligands. In contrast to the binding of FH to surfaces, which prevents further C3b generation and deposition (negative regulation), the binding of the FHRs enables C3b amplification to proceed unhindered. This competition with FH would be influenced by the concentration and composition of the FHRs relative to FH at the site of complement activation, the density and relative deposition of C3b, iC3b, and C3dg, the carbohydrate and polyanion composition, and the presence of additional ligands on the complement-activating surface. Understanding all of these factors will help to understand the association of the FHRs with disease.

Competition between FH/FHL-1 and FHRs on host and altered host surfaces or molecules

The main function of FH/FHL-1 is to control the AP amplification loop and to prevent tissue damage by accidental complement activation on self surfaces. Basically, the role of FH is to maintain the density of C3b molecules on host surfaces below a critical threshold, because if this threshold is exceeded, C3b amplification proceeds without control and tissue damage occurs. A first consideration

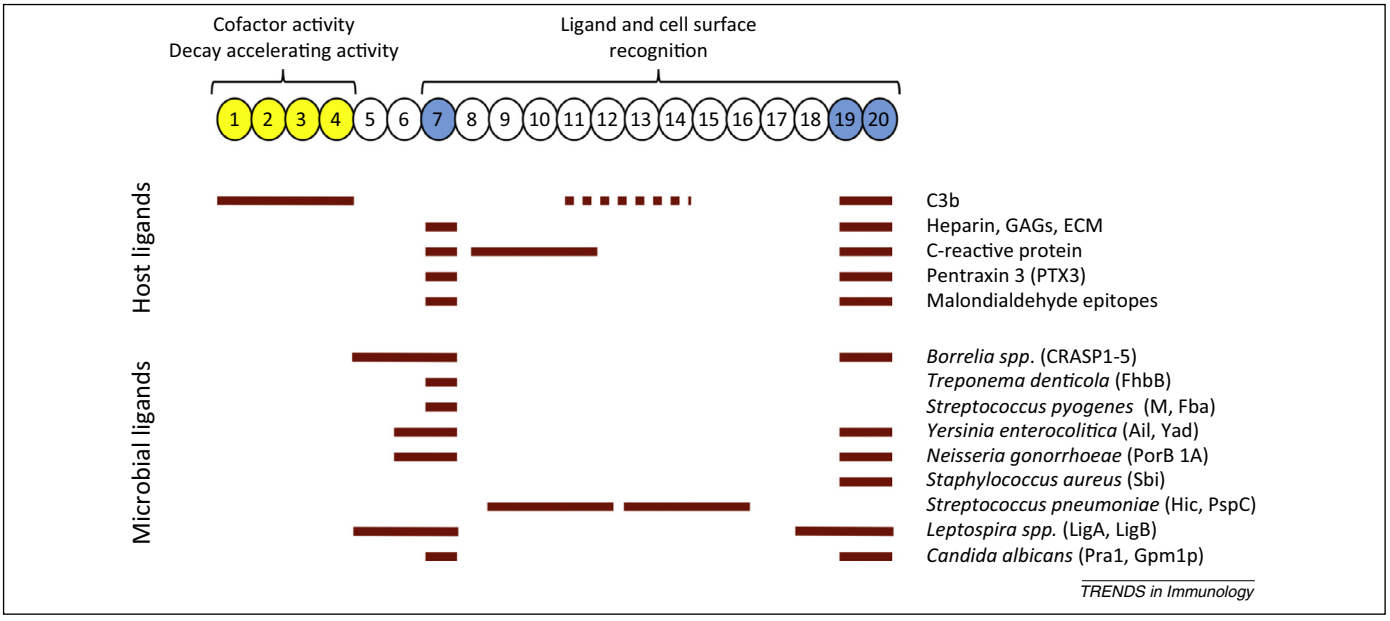

Figure 2. Main binding sites within factor H (FH) for host and bacterial ligands. Figure depicts a diagram of the 20 short consensus repeat (SCR) domains of FH. SCRs 1–4 are responsible for the cofactor and decay-accelerating activities (yellow) and SCRs 7 and 19/20 contain major recognition sites for host and bacterial ligands (blue). The main host ligands are listed and their binding sites in FH indicated by red horizontal lines. Selected microbial ligands are similarly shown in the bottom part of the figure.

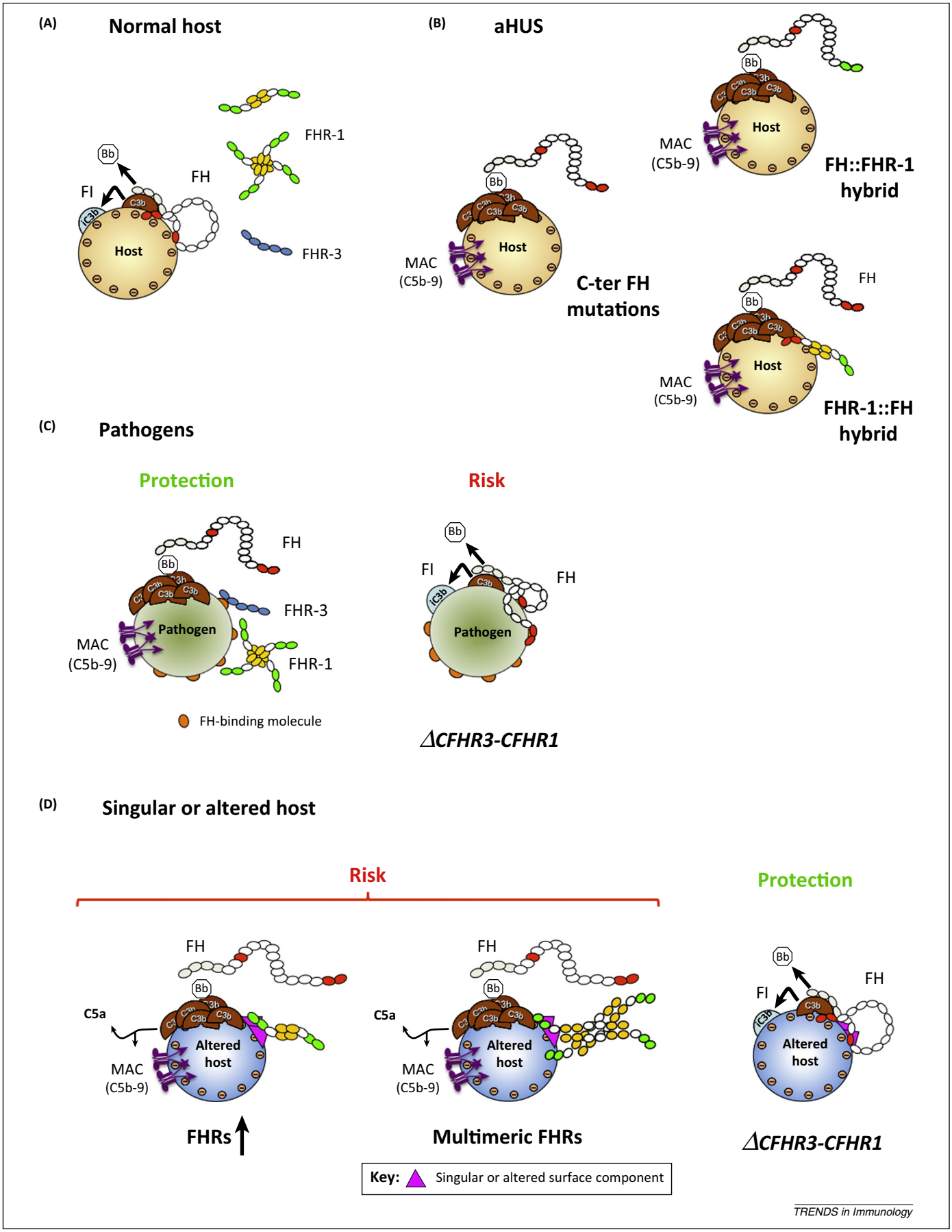

Figure 3. Competition between factor H (FH) and factor H-related (FHR) proteins on self, altered-self, and pathogen surfaces. The figure depicts potential scenarios of FH/FHR competition. For host cells (**A**), available data suggest that, on normal endothelial cells, there is no competition between FH and FHR-1 and FHR-3. In atypical hemolytic

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regarding the postulated competitor role of the FHRs is what prevents them from inhibiting the function of FH protecting the normal host cell surfaces and causing disease. A likely answer to this question is that, despite the very high conservation of the FH surface recognition domains in some of the FHRs (Figure 1), competition on normal host tissues is very limited. This view is supported by observations in aHUS patients indicating that exchanging the C-terminal regions between FH and FHR-1 has pathogenic consequences identical to those of the disease-associated FH mutations that disrupt the C-terminal functionalities (Figure 3A,B). Therefore, despite there being only two amino acid differences (S1191L, V1197A) between the C-terminal regions of FH and FHR-1, these differences are sufficient to alter sialic acid recognition [58], conferring distinct surface-binding specificity to FH and FHR-1 and eliminating the risk of undesirable competition between them for host tissues. The possibility that the FH/FHR competition affects mainly a specific subset of surfaces that does not include normal host surfaces may help to understand the association of the FHRs with pathology. Our proposal is, therefore, that the FHRs originated through evolution to prevent binding of FH to certain pathogens (Figure 3C), and that they also compete with binding of FH to altered host surfaces (Figure 3D), perhaps because these altered host surfaces include molecules that resemble those present on the surface of pathogens. This proposal assumes that there must be complement activation and C3 deposition on these surfaces (spontaneous, following a trigger, or both) that would be accelerated in the presence of these FHR proteins. The presence of the activated C3 fragments iC3b and C3dg, which are better ligands for some FHRs than for FH [10,11], may be crucial to sustaining competition between FH and the FHRs at these surfaces. Additional, poorly characterized 'injury-associated' changes in host surfaces may favor this competition [59].

In this setting, the relative amounts of FH and FHRs, established by their levels of expression or activity, appear critical to the modulation of complement regulation and to determining susceptibility to complement-mediated injury. Plasma levels of FH vary widely (116–562 μg/ml) in humans as the result of the combined effect of genetic and environmental factors. Notably, 63% of the variation in plasma levels of FH is determined genetically by polymorphisms probably linked to the *CFH* gene in 1q32 [60]. Strong linkage disequilibrium limits the genetic variability in that genetic region to a few *CFH–CFHR3–CFHR1* extended haplotypes [2,17]. Interestingly, two of these haplotypes that have been strongly associated with various diseases determine significantly different plasma levels of FH and FHRs. Haplotype H3 determines significantly lower levels of FH in plasma and may also affect FHR-3 levels in plasma (Bernabeu-Herrero *et al.*, submitted). This could imply increased competition between FH and FHR-3 and may explain the association of this haplotype with protection against meningococcal disease [60]. Haplotype H4 carries the *CFHR3–CFHR1* deletion and also determines increased levels of FH [61], a combination that, enhancing FH regulation on surfaces, could explain the association of this haplotype with protection against AMD and IgAN [2,17].

In addition to these genetically determined variations [60], some reports suggest that FH/FHR protein levels may also be actively regulated on infection [62,63] and there is evidence that FHRs levels may also change in other situations (our own data) [64,65]. Changes in FHR levels may explain the episodic nature of some diseases associated with complement dysregulation in which relapses often associate with infection (i.e., C3G, IgAN). The possibility that FHR levels may be influenced by physiological and pathological conditions warrants further studies since it would be predicted that a local increase in the concentration of the FHRs would, through enhanced FH competition, enable (rapid) enhancement of complement activation.

The FH/FHR balance may also be altered in situations in which the FHRs are mutated and acquire gain-of-function activities. Hybrid and mutant FHR-1, FHR-2, or FHR-5 proteins containing a duplicated dimerization domain are good examples of this situation [10,11,14]. Similarly, the association with C3G of novel *CFHR3::CFHR1* and *CFHR5::CFHR2* hybrid genes may be explained by an altered FH/FHR balance [66]. These hybrid genes express the hybrid protein in addition to the FHRs produced by the normal copies of *CFHR1* and *CFHR3*, which result in increased levels of these FHRs to compete with FH.

The identification of the specific surfaces that sustain the FH/FHR competition in these pathologies is, however, challenging. The GBM and Bruch's membrane (BM) are good candidate surfaces for competition between FH/FHL-1 and FHRs. It has been shown that FH, FHL-1, FHR-1, and FHR-5 bind to components of the extracellular matrix (ECM), the major constituent of the GBM and BM, and that FHR-5 inhibits the surface-associated cofactor activity of FH by competing with its binding to the ECM [67–69]. This competition may increase by the presence of microbial ligands or other physiological or pathological substances deposited together with C3 activated fragments along the GBM or BM.

There are additional molecules on apoptotic cells, retinal deposits, and other structures that can sustain this FH/FHR competition. One such ligand is malondialdehyde (MDA) epitopes, which originate from peroxidation of membrane lipids due to oxidative stress [70]. It is thought that MDA epitopes contribute to the recruitment of FH to

uremic syndrome (aHUS) (**B**), it has been established that the pathogenic mechanism is a defect in the protection of endothelial cells from complement damage. The prototypical genetic defects are C-terminal FH mutations and hybrid proteins involving FH, FHR1, and FHR3. The pathogenicity of the latter demonstrates both that FHR-1 and FHR-3 cannot replace the complement regulatory activities of FH and that they do not compete with the binding of FH to aHUS-relevant surfaces. Certain pathogens (**C**), like *Neisseria*, express FH-binding proteins on their surface that contribute to the survival of the pathogen. Different FH/FHRs ratios could explain differences in the susceptibility of hosts to *Neisseria* infection. Alteration of the FH/FHR ratio and modification of host surfaces (**D**) by genetic or environmental factors potentially contribute to sustaining competition between FH and the FHRs and result in pathology. Singular and altered host surfaces are terms used here in a wide and overlapping sense to refer, for example, to extracellular matrix and other cell surface components modified by aging, microbial or chemical agents, or deposition of immune complexes (including those containing galactose-deficient IgA) or even to iC3b or C3dg opsonized surfaces. We would like to suggest that on these singular and altered host surfaces an unbalanced FH/FHR ratio causes complement dysregulation.

the surface of apoptotic cells, where FH neutralizes their proinflammatory properties and halts complement activation [71]. In AMD, a common eye condition among elderly people, this situation may be particularly relevant as in the retina dying cells are continuously generated and must be efficiently removed. FH binds MDA epitopes through SCRs 6/7 and 19/20, which may explain the association of both the 402His polymorphism in SCR7 with increased risk and the *CFHR3–CFHR1* deletion with strong protection against AMD [17,72–75]. Accordingly, the FH risk variant 402His was shown to exhibit decreased binding to MDA compared with the 402Tyr variant and evidence was provided suggesting that FHR-1 could compete with the binding of FH to MDA [71].

IgAN is a common form of primary glomerulonephritis characterized by galactose-deficient IgA1 (Gd-IgA1)-containing immune complexes that deposit in the glomerular mesangium producing progressive kidney disease [76]. IgAN is also strongly associated with the *CFHR3–CFHR1* deletion [16]. Similarly to AMD, the reported protection against IgAN conferred by the lack of FHR-3 and FHR-1 may also relate to the generation of altered host surfaces. The pathogenesis of IgAN is currently modeled as a sequence of multiple events, one being the generation of antiglycan antibodies that recognize GalNAc-containing epitopes on Gd-IgA1 [77]. It is possible that Gd-IgA1-containing immune complexes deposited in the mesangium may resemble pathogen surfaces where competition between FH and FHRs may occur. We speculate that this competition would be further enhanced by the deposition of the C3 activated fragments generated by activation of the lectin pathway [78]. The advantage of individuals carrying the *CFHR3–CFHR1* deletion would be having less FHR to interfere with the regulation by FH of the complement activation induced by these Gd-IgA1–antiglycan immune complexes in the kidney.

Complement also plays an important role in SLE, a severe autoimmune disease characterized by the presence of autoantibodies that result in tissue injury of multiple organs [24]. Here, in contrast to AMD and IgAN, the *CFHR3–CFHR1* deletion represents an important predisposition factor, suggesting that in this case decreased FH/FHR competition is deleterious. Two possibilities have been proposed to explain this association. One is based on the capacity of FHR-1 to inhibit C5 convertase activity and membrane attack complex (MAC) formation, a role of FHR-1 that is currently controversial. The second explanation relates to the possibility that carriers of the *CFHR3–CFHR1* deletion generate autoantibodies against FH, similar to the situation in aHUS where these antibodies are associated with homozygous deficiency of FHR-1 [79]. The contrasting phenotypic association of SLE with the *CFHR3–CFHR1* deletion, opposing the protective effect observed for IgAN and AMD, may also suggest that some AP activation could be beneficial in SLE. For example, the *CFHR3–CFHR1* deletion may result in less opsonization and opsonophagocytic removal of autoantigens due to increased complement regulation by FH on apoptotic cells. In that setting, enhanced levels of autoantigens may promote the production of pathogenic autoantibodies recognizing these targets.

Concluding remarks

As a whole, there is little convincing evidence for the existence of physiologically relevant complement inhibitory activities of the FHRs. However, recent data and critical evaluation of previous studies suggest a FH antagonistic function for the FHRs, termed complement deregulation. Future studies will define the ligands and surface specificity of the FHRs in more detail and also the conditions under which competition with FH occurs. This knowledge will advance our understanding of the pathogenic mechanisms of several diseases associated with complement dysregulation. We do not exclude that FHRs may have functions independent of the activity of FH, such as the enhancement of complement activation, as showed for FHR-4, and even some complement inhibitory activities. Currently, however, strong *in vitro* evidence and data from various diseases suggest a major role of the FHRs as competitive inhibitors of FH for fine-tuning the discrimination of complement activating surfaces.

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