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Review

Attachment of the soluble complement regulator factor H to cell and tissue surfaces: relevance for pathology

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Summary. Complement is a central element of innate immunity and this vital defense system initiates and coordinates immediate immune reactions which attack and eliminate microbes, foreign particles and altered self cells. Newly generated activation products are extremely toxic and consequently, activation is highly restricted in terms of time and space. The initial activation of the alternative complement pathway occurs continuously and the early phase acts indiscriminatoryl and forms on any surface. However, the system discriminates between self and foreign, and therefore allows activation on foreign surfaces e.g. microbes, and restricts activation on host cells. Consequently, self cells and tissues are protected from the harmful activation products. This protection is mediated by specific regulators or inhibitors, which exist in the fluid phase and/or in membrane-bound forms. Here we review a novel mechanism, i.e. the attachment of the soluble complement regulator factor H to the surface of self cells. This attachment, which is demonstrated experimentally by means of immunofluorescense microscopy and by flow cytometry, increases the inhibitory potential at the cell surface and mediates protection by reducing the local formation of toxic inflammatory products. This attachment is highly relevant and has pathophysiological consequences in several human diseases, including Factor H-associated hemolytic uremic syndrome (FH-HUS), membrano-proliferative glomerulonephritis type II, recurrent microbial infections and chronic inflammation, e.g. rheumatoid arthritis and immune evasion of tumor cells. Defects of this safeguard activity have been recently understood in patients with FH-HUS. Point mutations in the Factor H gene occurring in the C-terminus of the protein result in impaired cell binding capacity of Factor H and, consequently, during an inflammatory insult endothelial cells are not properly protected and are damaged.

Key words: Immune defense, Innate immunity, Hemolytic uremic syndrome, Disease mechanisms, Cellular binding

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Complement: activation and regulation

Complement is a central part of the innate immune system, forms a barrier and functions as a true safeguard system in order to maintain tissue integrity (Walport 2001a,b). The complement system is composed of approximately 40 proteins which are either integral membrane proteins or soluble plasma proteins. Similar to the blood clotting system, inactive components circulate in plasma and are activated in a cascade-type manner. The fully activated system has deleterious effects: it opsonizes particles, modified or foreign cells and consequently favors phagocytosis; and, in addition, creates a toxic membrane attack complex which forms holes in the membranes and causes lysis of attacked cells (Morgan and Harris, 1999). Due to these deleterious effects the activation of this vital defense system is tightly regulated. This tight control is reflected on one hand by the large number of inhibitors and on the other hand by the redundant and overlapping activities of the individual regulators. About half of the identified complement proteins represent regulators or inhibitors, which keep the system in track. These regulators control activation at almost every level; particularly the initial steps of the cascade are efficiently regulated and thus activation is limited both in time and space.

The complement cascade is initiated by three distinct pathways, the alternative, the classical and the lectin pathway. The classical and lectin pathways are initiated by specific molecules, such as antibodies and carbohydrates. The alternative pathway, which the evolutionary oldest pathway, represents an immediate safeguard system. The alternative pathway is continuously activated, and the initial activation, which occurs at low levels, is indiscriminatory and acts on any

type of surface, i.e. self cells and tissues, as well as foreign cells or particles. Activation proceeds unrestrictedly on foreign cells, but is actively downregulated on self cells. Self cells are equipped with membrane-bound regulators and in addition they attach fluid-phase regulators to their surface in order to increase the inhibitory activity. In contrast to this, unrestricted activation of the complement cascade occurs at the surfaces of foreign particles, most microbes and altered self cells, such as tumor cells, as these cells either lack endogenous regulators and attachment structures, or they express endogenous regulators at lower numbers. The fully unrestricted, uncontrolled activation generates a battery of damaging products, leading to the elimination of the target.

Factor H, a soluble complement regulator

Activation of the complement cascade is tightly controlled. A large number of regulators act on the level of C3, which is the central molecule and a common component of all three complement pathways, and a common starting point for the terminal pathway. Conventionally the inhibitors are grouped according to their site of action or distribution: plasma regulators act in the fluid phase and membrane proteins act directly on cell surfaces. An additional discrimination of the regulators is based on their function and the substrates which they control. Each activation pathway, the alternative, the classical and the lectin pathway employs specific regulators and all three pathways merge at the level of C3 and C3 convertase, explaining the overlapping activities of several inhibitors. Regulators acting on the level of C3 or C4 (also termed C3b/C4b binding regulators) are related in terms of structure and evolution. The fluid-phase regulators of the alternative pathway, Factor H and FHL-1, and of the classical pathway, C4BP, are structurally related and most likely precede from a common ancestor (Kemper et al., 1998; Krushkal et al., 2000). In addition, membrane-bound regulators are complement receptor type 1 (CR1, CD35) and they decay accelerating factor (DAF, CD55) and membrane cofactor protein (MCP, CD46). These regulators have overlapping activities and control formation, stability and degradation of C3b or C4b and of the C3b or C5b generating convertases. These inhibitors display cofactor activity (except for DAF) for the serine protease factor I in the degradation of C3b. As C3b is generated by all three pathways, and is essential for the terminal activation process, this molecule acts as a hub, a central focus point during complement activation. In addition, the regulators show decay acceleration activity (except for MCP), they inhibit the assembly and enhance the decay of the alternative or the classical pathway C3 convertases C3bBb, C4bC2a and also of the C5 convertases (Table 1). Expression of membrane-bound regulators, however, is restricted. CR1 is expressed on erythrocytes, eosinophils, monocytes, macrophages, neutrophils, B- and some T-cells, glomerular podocytes, endothelial cells, follicular dendritic cells and mast cells (Klickstein and Moulds, 2000). MCP is expressed on neutrophils, monocytes, platelets, lymphocytes, granuolocytes and endothelial and epithelial cells (Liszewski and Atkinson, 2000) whereas DAF is found on erythrocytes, all leukocytes and platelets (Friese et al., 2000; Kuttner-Kondo et al., 2000) (Table 1). In this review we focus on the recently reported cell attachment activities of the soluble alternative pathway complement regulator factor H and describe the physiological and pathophysiological consequences of this novel activity.

Factor H is a multifunctional regulator

The human plasma protein Factor H is the central fluid-phase regulator of the alternative complement pathway (Zipfel et al., 1999). The protein is 150 kDa in size, it is composed of 20 individually folding protein domains termed short consensus repeats (SCRs) and is a potent suppressor of the alternative complement pathway. As a complement regulator, Factor H maintains tissue integrity and possesses anti-inflammatory

Table 1. Complement regulators acting on the level of C3 and C4.

REGULATOR	PATHWAY	COFACTOR ACTIVITY	DECAY ACCELERATION	EXPRESSION
Soluble				
Factor H	AP	+	+	liver tissue, endothelial cells, monocytes, fibroblasts
FHL-1	AP	+	+	liver tissue, endothelial cells
C4BP	CP	+	+	hepatocytes
Membrane bound				
CR1 (CD35)	AP/CP	+	+	erythrocytes, eosinophils, monocytes, macrophages, neutrophils, follicular dendritic cells, B-cells, a subset of T-cells, glomerular podocytes, endothelial cells, mast cells
MCP (CD46)	AP/CP	+	-	neutrophils, monocytes, platelets, lymphocytes, granuolocytes, endothelial cells, epithelial cells
DAF (CD55)	AP/CP	-	+	erythrocytes, all leukocytes, platelets

activities. However, this multifunctional protein displays additional activities, such as cell attachment activity for monocytes and serves as an adhesion ligand for neutrophils (Nabil et al., 1997; DiScipio et al., 1998). It also acts as a ligand for osteopontin, bone sialoprotein and adrenomedullin (Fedarko et al., 2000; Pío et al., 2001; Jain et al., 2002; Martínez et al., 2003). These diverse functions are mediated by distinct domains, which are scattered throughout the factor H molecule.

Sources of factor H

Similar to most complement proteins, the plasma protein factor H is synthesized and secreted by liver cells. In addition other cell types and tissues do also contribute to local factor H levels (Friese et al., 1999, 2000, 2003; Schlaf et al., 2001). Of particular interest are platelets, which contain factor H in their α granules and release this complement regulator upon activation (Devine and Rosse, 1987; Devine et al., 1987). Importantly, endothelial cells also produce factor H, and this production is regulated by inflammatory cytokines (Friese et al., 1999; Schlaf et al., 2001).

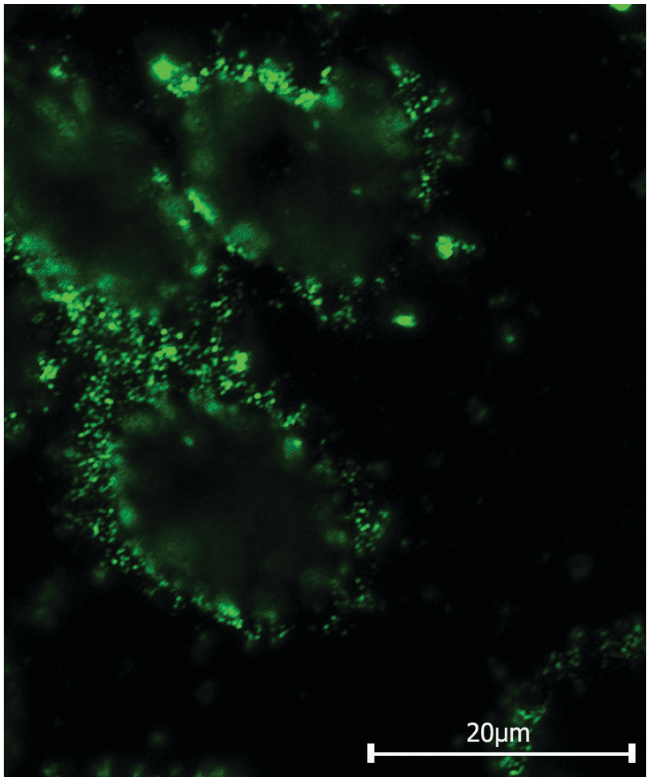


Fig. 1. Binding of Factor H to endothelial cells: microscopic analysis. Human umbilical vein endothelial cells (HUVEC) are grown in the absence of serum in microchamber slides. After incubation with purified FH, factor H-specific antibody and labeled secondary antibody are added.

Interaction of Factor H with cell surfaces

Factor H is a plasma protein which binds to cell surfaces via specific cellular receptors, e.g. integrins (CD11b/CD18, i.e. CR3) (DiScipio et al., 1998), and also via general polyanionic cell surface structures, such as proteoglycans, sialic acids, heparansulfate chains or glycosaminoglycans (Meri and Pangburn, 1990; Hellwage et al., 2002). Although Factor H is a plasma protein and considered to act in the fluid phase, surface binding and a role of sialic acids in this interaction was reported long ago (Kazatchkine et al., 1979; Carreno et al., 1989).

Apparently, binding of Factor H to cell surfaces is a rather complex phenomenon, the protein has three distinct heparin binding sites which are located in or close to SCR7, SCR13 and SCR20 (Pangburn et al., 1991; Blackmore et al., 1996, 1998; Ram et al., 1998). This interaction seems of central importance for discrimination between activator and non-activator (i.e. polyanion rich) surfaces. Thus, binding of the fluid-phase regulator Factor H to cell or tissue surfaces is relevant for protection of endogenous cells, membranes and surfaces. Although downregulation of the activated complement cascade is modulated by several regulators, a particular, specific role is concluded for Factor H. Factor H dysfunction correlates with a wide range of distinct human diseases, including Factor H-associated hemolytic uremic syndrome, membranoproliferative glomerulonephritis type II, recurrent microbial infections and chronic inflammation, e.g. rheumatoid arthritis, and immune evasion of tumor cells.

Experimental evidence for factor H binding to cells

Recent assays have used novel approaches to address the binding of Factor H to human cell surfaces and the physiological relevance of this interaction.

Binding of fluid-phase Factor H to human umbilical vein endothelial cells (HUVEC) is visualized by immunofluorescence microscopy (Fig. 1). The attached regulator is evenly distributed over the complete cell surface. This interaction, however, is not restricted to the cell membrane, the protein also covers the extracellular space between the individual cells.

Binding of Factor H to the surface of endothelial cells is also visualized by *flow cytometry*. Dose-dependent, specific binding of Factor H is shown at factor H concentrations ranging from 1 to 10 μg and reaches an equilibrium at the highest concentrations tested (Fig. 2A). The actual plasma concentration of factor H is approximately 500 μg/ml, suggesting that cells which are in direct contact with plasma are completely covered with the regulator. The use of a domain-mapped monoclonal antibody, namely C18, which binds to a specific epitope within SCR20, strongly reduces binding, resulting in almost complete inhibition. This effect demonstrates that a single, C-terminal domain of Factor H mediates surface binding to

endothelial cells (Fig. 2B) (Oppermann et al., submitted). These results clearly show a cell binding activity of the human serum protein Factor H and suggest a protective role of this soluble protein at the endothelial cell surface.

Site of action

Polyanions are found on all self cells, thus surface binding of Factor H is a rather general phenomenon and is not restricted to endothelial cells. The tolerance of any given cell towards complement attack depends on the number and activity of membrane-anchored and surface-attached regulators, which act on or close to the cell surface. Thus, tissue cells as well as circulating cells use membrane-anchored regulators such as CR1, DAF, MCP and CD59 and based on their surface proteoglycan composition they can attach additional fluid-phase regulators such as plasma Factor H. Cells in direct contact with plasma are sufficiently coated with Factor H and, in addition, local synthesis of this regulator, as occurs in the synovium and in endothelial cells, contributes to cell protection. This scenario may explain the high sensitivity of the kidney glomerular basement membrane during stress that leads to strong complement activation. The experimental data show that Factor H is deposited on the surface of cells and within the extracellular matrix and thus provides an additional shield against complement attack (Figs. 1, 2). The interaction with cell surface structures is mediated via the C-terminus of the protein and the initial contact exposes the complement regulatory N-terminal domain (Pangburn, 2002; Oppermann et al., submitted, Fig. 2B).

Functional consequence: activity in direct vicinity to surfaces

This reported cell binding activity provides novel perspectives to explain the role of the soluble complement regulator Factor H for protection of self cells. In addition to its activity in the fluid phase, Factor H binds to cell surfaces and to molecules which form the extracellular matrix. The bound inhibitor maintains its complement regulatory activities. This scenario shows that complement control occurs at two layers. The inner layer activity, i.e. directly at the cell membrane is mediated by membrane-bound regulators, such as DAF and MCP. A second, outer layer is further apart and mediated by surface-attached soluble regulators, such as factor H. The extended factor H molecule has a length of ca. 80 nm (Perkins et al., 2002), and given this size and the flexibility of the protein, the extended, surface-attached regulator can cover and control a rather large area (Fig. 3).

Surface-attached Factor H forms an effective and strong barrier in the direct vicinity of, or close to the cell membrane. At this location the surface attached inhibitor decreases the number of newly generated C3b molecules and thus reduces the number of membrane-deposited C3b, thereby limiting locally the progression of the complement cascade. This activity seems relevant for any cell and surface, but is of particular importance for cells which express a low number of membrane regulators, or for tissues which completely lack endogenous membrane regulators, such as the kidney glomerular basement membrane (Hogasen et al., 1995).

The pivotal role of Factor H acting on cell surfaces is demonstrated for mutant factor H proteins observed in patients suffering from Factor H-associated hemolytic uremic syndrome (FH-HUS). Proper activity of the multifunctional serum protein Factor H is central for endothelial cell integrity and dysfunctions and mutations of the protein are related to the development of the disease (Sánchez-Corral et al., 2002; Manuelian et al., 2003).

Disease association

Apparently under normal conditions the concerted action of membrane-anchored and surface-attached fluid phase regulators provides sufficient means for control and for protection of host cells from the activated complement system. However, during inflammation or microbial infection complement activation occurs

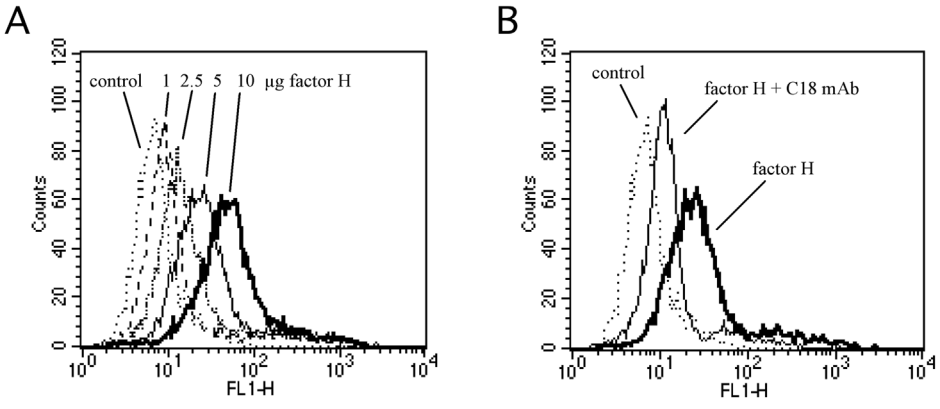
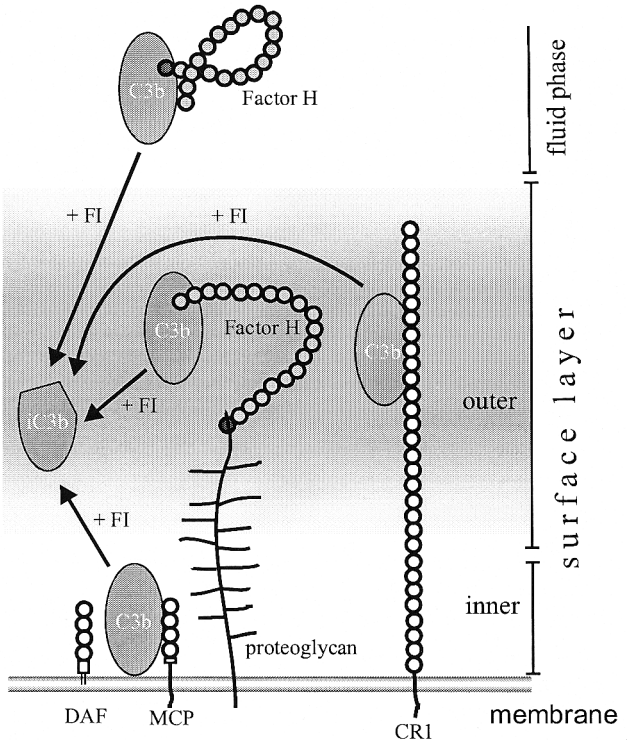


Fig. 2. Binding of Factor H to endothelial cells: flow cytometry. **A.** HUVEC cells are incubated with the indicated concentrations of purified FH and binding is detected upon treatment with FH-specific antibody, followed by FITC-labeled secondary antibody. **B.** Binding of FH to the cells is inhibited by mAb C18, which recognizes an epitope within the most C-terminal SCR20. Detection is the same as in A. x- and y-axes correspond to the relative fluorescence intensity and the number of cells, respectively.



rapidly and heavily and activation needs to be restricted in space e.g. to the bacterial surface. At that time neighboring host bystander cells must protect themselves from the deleterious effects of the activated complement system and the cells need to downregulate complement activation very efficiently. Under these conditions acquisition of additional, effective regulators from the fluid phase enhances protection and for a tissue cell in close proximity to a complement-activating microbe this step can be a matter of life or death. In this regard,

Fig. 3. Different layers of complement control at the endothelial surface. Complement control occurs on different layers: at and close to the surface, and in the fluid phase. Based on the size and composition of various regulators the surface layer is divided into an inner layer which is represented by small-sized membrane-integrated complement regulators DAF and MCP and which control complement activation in the direct vicinity of the membrane. Based on the size of these proteins this layer covers a distance of approx. 20 nm. The outer phase of the surface layers is represented by CR1 which extends from the cell membrane by ca 120 nm and by proteoglycan-attached regulators such as factor H, which is derived from the outer fluid phase. The proteoglycan binding site of factor H located in the most C-terminal SCR is indicated by the black domain. The outer surface layer controlled by factor H is indicated with the grey color. As it controls complement activation in the direct vicinity of the cell membrane this layer acts as an additional shield, which decreases the number of C3 activation products formed directly on the membrane.

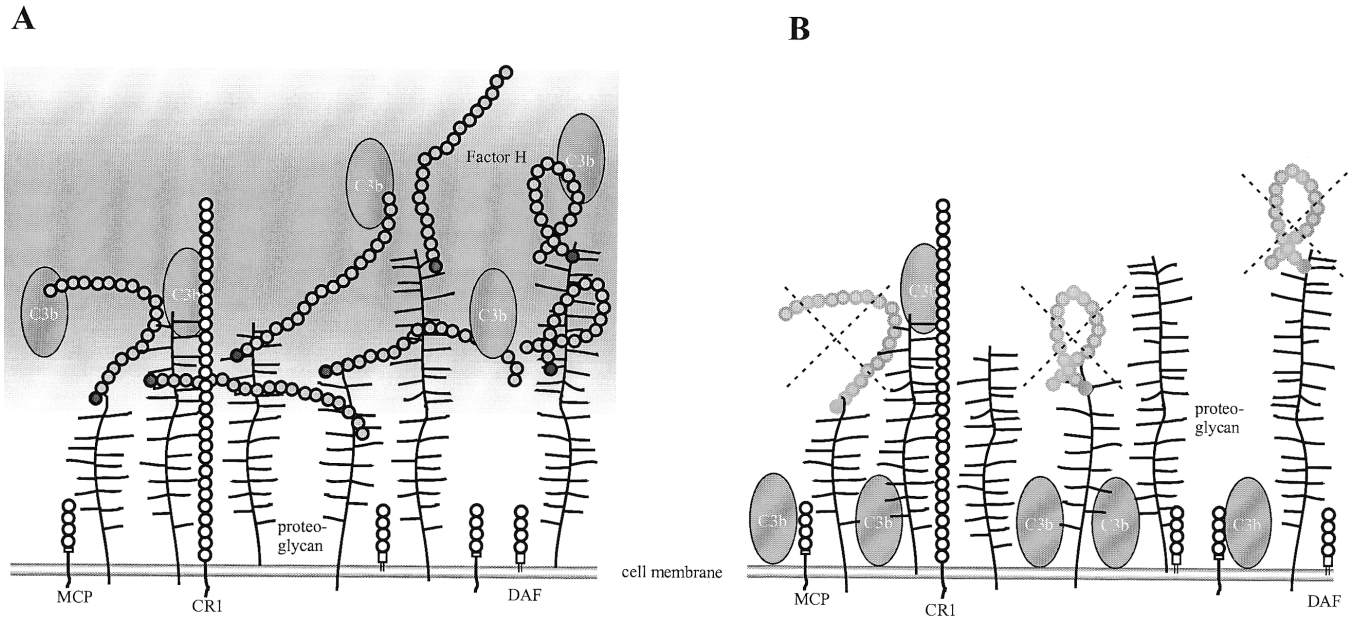


Fig. 4. Proposed effect of factor H binding to the endothelial surface. **A.** Complement control at the endothelial surface. The complement control mediated by the membrane-anchored regulators (e.g. DAF, MCP and CR1) is further assisted by factor H bound to proteoglycans present as membrane proteins or in the extracellular matrix. The outer surface layer controlled by factor H is indicated with the grey color. This full activity of membrane regulators and the surface-attached soluble regulator factor H protects the cell from complement attack and in the vicinity of activation decreases the number of C3b activation products which make their way directly to the cell membrane. **B.** Scenario with factor H proteins carrying mutations, which affect polyanion binding, as has been reported in patients with atypical hemolytic uremic syndrome. Factor H with mutation(s) affecting the polyanion interaction site at the C-terminus shows reduced binding to cell surface proteoglycans. This step results in a lower complement regulatory activity in the vicinity of the cell and, consequently, enhances C3b deposition and complement activation on the cell membrane.

acquired Factor H forms a second barrier like an aura around the cells (Figs. 3, 4), which limits the number of surface-deposited C3b molecules.

These theoretical considerations indicate a role of the multifunctional protein Factor H for a wide range of diseases, and different outcomes of the various disease settings are based on the plasma concentration of functionally active Factor H, as well as the contribution or redundant activities of additional local regulators. The scenario outlined here is actually relevant for pathophysiological settings, as Factor H expression is increased during inflammatory reactions and is responsible for the pathophysiology of several human diseases, including hemolytic uremic syndrome, membranoproliferative glomerulonephritis type II, recurrent microbial infections and chronic inflammatory conditions, such as rheumatoid arthritis and immune evasion of tumor cells, as well as disorders in the coagulation system. Increased levels and expression of Factor H and of the Factor H related FHL-1 protein have been reported in synovial fluid and synovial fibroblasts derived from patients suffering from rheumatoid arthritis (Friese et al., 2000, 2003) and in tumor cells (Junnikkala et al., 2000, 2002) as well as for endothelial cells (Friese et al., 1999; Schlaf et al., 2001). In addition, binding of Factor H to platelets, neutrophils and endothelial cells has been reported (Devine and Rosse, 1987; DiScipio et al., 1998; Manuelian et al., 2003).

FH-HUS

Recently the involvement of complement and genetic predisposition in a subset of patients with atypical HUS (FH-HUS) has been described (reviewed in Taylor, 2001; Zipfel, 2001; Mathieson, 2002). Low serum levels of the central complement component C3 and in several cases also FH has been described in several HUS patients (Landau et al., 2001; Caprioli et al., 2001). The consumption of C3 is due to uncontrolled complement activation via the alternative pathway. Lack of functionally active plasma Factor H caused this defect (Noris et al., 1999; Thompson and Winterborn, 1981; Pichette et al., 1994; Warwicker et al., 1999). Further genetic studies showed a clustering of Factor H gene mutations in the most C-terminal domain of the protein, namely within SCR20 (Caprioli et al., 2001; Pérez-Caballero et al., 2001; Richards et al., 2001; Zipfel, 2001). This domain includes the binding sites for C3b/C3d and heparin and makes the initial contact with the ligands, therefore it is central for cell binding and host recognition (Hellwage et al., 2002; Oppermann et al. submitted). In recent studies Factor H proteins with mutations in SCR20, either directly purified from patient plasma or the recombinant mutant proteins, show functional defects. Mutants W1183L, V1197A and R1210C had reduced affinity to C3b in an ELISA assay (Sánchez-Corral et al., 2002) and the E1172Stop, R1210C and R1215G mutants displayed severely reduced binding to immobilized C3b, to heparin and to endothelial cells (Manuelian et al., 2003). These studies demonstrate a role of the plasma protein Factor H for endothelial cell protection and show that the defective protein functions relate to microvascular damage. This activity is particularly important for endothelial cell surfaces and/or platelets, and thus, at sites of inflammation, dysfunctional Factor H may result in reduced complement control. Apparently, the vast majority of FH-HUS patients are at an adult age and are heterozygous for the mutations. In contrast to this, homozygous mutations, which are shown by the absence of Factor H in plasma, are associated with membrano-proliferative glomerulonephritis type II and occur early in life.

Absence of Factor H in plasma results in membranoproliferative glomerulonephritis type II. This effect has been demonstrated in humans (Ault et al., 1997), also for a patient who developed auto-antibodies against Factor H, which bind to the complement regulatory domain within SCR3 (Meri et al., 1992; Jokiranta et al., 1999), in Factor H-deficient pigs (Hogasen et al., 1995) and also in Factor H knock-out mice (Pickering et al., 2002). Apparently, lack of Factor H in plasma is – at least to some extent – substituted by other regulators, such as the related FHL-1 serum protein and the membrane-bound regulators. However, the glomerular membrane lacks endogenous regulators and thus, when plasma Factor H is absent, continuous and permanent complement activation occurs at this particular site and C3 is deposited, resulting in membrane thickening and, consequently, in defective filtration. In general terms this disease occurs early in life and Factor H-deficient patients show damaged kidneys within 1 to 2 years of life. The molecular mechanisms of the disease have been deciphered for one human and for the pigs. Genetic analyses show framework mutations in two Cys-residues within SCR9 and SCR16 for the human and of a non-framework I1166R residue in SCR20 for the pigs (Ault et al., 1997; Hegasy et al., 2002). In both cases the mutations affect protein release. mRNA transcripts are detected inside the cells, protein synthesis occurs and the protein is present within the cells. However, a block of protein secretion causes intracellular accumulation and consequently the protein is absent in plasma.

Overexpression of Factor H and in some cases also of FHL-1 has been reported under chronic disease settings, such as rheumatoid arthritis as well as in tumor cells. Synovial fibroblasts derived from rheumatoid arthritis overexpress the two complement regulators which have been demonstrated on the mRNA and protein level. Similarly, high concentrations of both regulators are detected in synovial fluid (Friese et al., 2003). Following secretion the released regulators attach to the surface of the secreting cells and to neighboring cells as well, and thus strengthen protection against the activated complement cascade. A similar mechanism is reported for some tumor cells, which also express, secrete and then attach the released regulators to their

surface in order to evade immune attack (Junnikkala et al., 2000, 2002).

Outlook

Data reviewed here show that Factor H plays a pivotal role in endothelial cell injury. This human plasma protein is the major soluble complement regulator, but also attaches actively to the cell surface and thus forms an effective layer for defense. This scenario suggests the existence of a second, additional layer of complement control close to or in direct vicinity of the cell surface, with Factor H forming a further protective barrier for complement activation in addition to the membrane-bound regulators (CR1, CD46, CD55, CD59).

Better understanding of the nature of this cellular interaction, the structure and function of Factor H and this process may be applicable and be of broader relevance for a number of diseases with reported endothelial cell dysfunction and disordered platelet functions, such as clotting diseases, thrombosis, inflammatory scenarios, hypertension, hyperlipidation, arteriosclerosis and tumor growth. Understanding these reactions will provide a basis for new approaches for diagnosis and therapy.

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