

## **Complement Factor H**

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**Veronique Fremeaux-Bacchi:** The complement system mediates a number of essential biological functions that participate in host defense against infection, initiation of the inflammatory reaction, processing and clearance of immune complexes and regulation of the immune response.

There are three pathways of complement activation. The classical pathway is initiated by Immune complexes; the lectin pathway by surface bound mannan binding lectin; and the alternative pathway by all the surfaces that are not specifically protected against it. Each generates a C3 convertase, a serine protease that cleaves the central complement protein C3, and generates the major cleavage fragment C3b.

Except for lysis elicited by the terminal C5b9 sequence, most of the biological effects derived from complement activation depend on ligand-receptor interactions between complement proteins and specific receptors on cells. For example: opsonization by C3b targets foreign particles for phagocytosis; chemotaxis by C5a attracts phagocytic cells; promoting antibody formation (breakdown of C3b generates a fragment (C3d) that binds to antigens enhancing their uptake by dendritic cells and B cells).

Binding of C3b to an activating surface of the alternative pathway is followed by the formation of a bimolecular complex with factor B, cleavage of factor B by factor D, and assembly of the alternative pathway amplification convertase C3bBb on the target surface. Once formed, the amplification convertase C3bBb cleaves C3 and generates C3b molecules which bind to the target surface.

Activation of the alternative pathways is precisely regulated. This is part due to the spontaneous dissociation and short half-life of enzymatic complexes and to the regulatory activity of a number of plasma and cell-associated proteins.

Two serum proteins factor H and factor I and two membrane proteins, CR1 (complement receptor type 1) and MCP (membrane cofactor protein), which interact with C3b, play a major role in the regulation of alternative pathway. These proteins inhibit the alternative pathway by regulating C3b deposition on the target.

Except for factor I, the genes encoding different regulatory proteins of Complement activation are located on the long arm of chromosome 1, a locus called RCA (Regulators of Complement Activation).

Inadequate regulation or extensive complement activation may alter the physiologic functions of normal cells and thus contribute to production of disease.

Complement protein factor H is the first regulatory protein of the alternative pathway. Factor H is a single-chain serum glycoprotein of 150 KD with a modular structure consisting of a tandem of 20 homologous units of about 60 amino acid,

called short consensus repeats (SCR). Factor H at the concentration of 500 microg/ml is one of the major complement proteins in plasma, second only to C3.

Factor H is the main representative of a family of related proteins. All proteins exhibit sequence similarities to factor H. FHL-1 (factor H-like protein 1) arises by alternative splicing from Factor H RNA and FHR (factor H-related) derived from different genes.

Numerous functional sites have been identified along the 20 SCR domain structure of factor H. Three C3-binding sites have been identified; in the SCR1-4 in SCR6-10 and SCR13-20.

Three polyanion binding sites like heparin and several glycoaminoglycans have also been identified in the SCR7, 13 and 20. FH displays anti inflammatory functions and acts as a ligand for CRP.

The AMD-associated polymorphism of FH is located domain 7, which is unique in that it can bind to heparin/heparan sulphate-containing surfaces and CRP.

Activation of complement by the alternative pathway displays unique features. Activation proceeds both in the fluid phase as well as on cell surfaces. In fluid phase, alternative pathway is continuously and nonspecifically activated at a slow rate in human plasma due to spontaneous hydrolysis of the highly reactive thioester bond of C3. "C3b like" form of C3 is capable of binding factor B resulting in formation of an initial C3 convertase. Free C3b is quickly inactivated by factor H.

The surface regulatory activity of FH is based on its ability to discriminate between the nonactivating "self" structures and "foreign" activator structures. In fact, factor H limits the effects of C3b deposition.

Factor H has two important functional domains that are located at the opposite ends of the protein. The N-terminal fragment of the factor H molecule is an essential fluid phase regulator of the alternative pathway. With the C terminal domain and SCR 7 factor H binds to cell and tissue surface and thus mediates its protective role also on host cell surface.

Activation of the AP is based on constant deposition of C3b on all surfaces. However amplification of the activation usually occurs only on foreign surfaces. Host cells are well protected if they possess polyanions like heparin, sialic acid, or GAG that facilitate binding of FH. FH inhibits the binding of factor B to C3b. Host cells are protected from the formation of amplification of C3 convertase.

On microorganisms lacking these regulatory elements, amplification proceeds with a efficient C3 convertase and the potential pathogen is rapidly covered with C3b which are ligand for receptors on phagocytic cells or participate to the formation of attack membrane complex C5b9 .

On non-activating surfaces the formation of the C3 convertase is inhibited by the preferential binding of factor H to C3b rather than factor B. Interestingly, recently genetic analysis identified a factor B haplotype which provide a protection from AMD. The binding of factor H for surface-bound C3b increases in the presence of

sialic acid or polyanions molecules. If SCR 19-20 is an important site for preventing AP activation of host cells, the SCR 7 of factor H also contributes to the recognition pattern.

Major problem is to clarify how C3b and H jointly recognize a nonactivator target and what is the fine specificity of this recognition. The local environment may play a critical modulator of this function.

AP regulation is at multiple levels and involves both inhibition of assembly or dissociation of assembled and functional C3 convertase. Factor H accelerates the decay of the C3 convertase by displacing bound Bb. Factor H acts as one of the factor I cofactors to degrade C3b. Inactivation of C3b by factor I generates iC3b that remains covalently linked to the target of complement activation but are unable to perpetuate complement activation.

Several membrane proteins like decay-accelerating factor (DAF), the C3b receptor (CR1, CD35) and membrane cofactor protein (MCP) inhibit formation of the alternative pathway C3 convertases on the cell surface thus contributing to the protection of host cells from damage by autologous complement.

Several studies have shown that bacteria, for example pneumococci, are able to bind factor H and upon binding factor H retained its cofactor activity in the presence of factor I. Acquisition of factor H at the surface inhibits AP activation and prevents opsonophagocytosis of the bacteria. An increasing number of important human pathogens express surface molecules which have been identified to interact with distinct regions of FH. It is a mechanism of immune evasion.

Dysregulation of the alternative pathway C3 convertase causes kidney disease. MPGN type II or dense deposit disease is a rare disease which is characterized by complement containing dense deposits within the basement membrane of the glomerular capillary wall. MPGN have been reported for patients who lack factor H in plasma and patients who are positive for auto antibody against amplification convertase. In the both cases there is a permanent activation of the C3 convertase and permanent C3 cleavage. Drusen observed in MPGN type II are identical to drusen in AMD.

Atypical haemolytic uraemic syndrome is a rare disease, which is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. The age of onset ranges from the neonatal period to adulthood. The majority of cases progress to end stage renal failure. The underlying pathology is thrombotic microangiopathy or TMA, a microvascular occlusive disorder of capillaries and arterioles.

Genetic analyses reveal a clear association between factor H and HUS. The frequency of factor H-associated HUS was established as 30 percent of cases. Up to 65 heterozygous substitutions have been reported. The mutations identified in the factor H gene are distributed over the factor H gene but most of the mutation cluster in the C terminal domains. The consequences of mutations are variable, including quantitative or functional deficiency involving ligand binding of C3b or heparin.

Three proteins, factor H, CD46, and factor I, implicated in the regulation of the complement alternative pathway are involved in the genetic predisposition to atypical HUS. This observation supports that the regulation of C3 cleavage plays a crucial role in the disease process.

There is four variants in factor H with potential functional effect. Several studies indicate that SNPs located in promotor region and in SCR 16 are exerting a unknown functional effect which results in an increased predisposition to HUS, the complement factor H histidine variant increases the chance of developing macular degeneration and was also associated with MPGN DD.

The pathogenetic mechanism that implicate factor H variants in AMD is not known yet.

What is the possible consequences of variants for the disease process and mechanisms of disease progression?

1. Locally, disruption of fine protection to the retina from C3b deposits with the time associated with a disproportionate complement activation could cause excessive production of C5b9 and contribute to the neovascular/exudative changes seen in neovascular forms of AMD.
2. Because complement activation is part of the immune response to microbial infection, is it possible that infectious etiologies play a role in macular degeneration?
3. According the implication of factor B and FH variants in the disease, the competition between factor B and factor H to C3b could be influenced by the variants of the both proteins?
4. Since the AMD-associated polymorphism of FH is located at domain 7 that binds heparin and CRP it might be that FH is needed locally in retina to prevent CRP-induced inflammation or in elimination C3b molecules.

In conclusion, AMD is a multifactorial disease. The majority of proteins involved in the AP are found in drusen. FH is a multifunctional plasma protein, which plays a major role in the regulation of AP, in the protection of cellular surface and presents anti inflammatory activity. There is evidence for the implication of local environment in the expression of the functional effect of the proteins. The AMD-associated polymorphism of FH is located domain 7 which is unique in that it can bind to heparin/heparan sulphate-containing surfaces and CRP. Factor H may act as both friend and foe. Factor H is capable to protect the tissue and its variant might also implicate in the chronic damage of retinal.

I thank the clinical investigators of Creteil University Eye Clinic and the members of the Department of Immunology of the Européan Georges Pompidou Hospital in Paris. Thank you very much.

Q: Tyrosine – so I guess it was an \_\_\_\_ agent – so an agent destroyed tyrosine, that the form of complement factor H that bound to this phenyl-Sepharose column was inactivated, so there were these two forms in the plasma at approximately equal concentrations. One fraction bound to phenyl-Sepharose column, the other didn't. If the bound fraction was treated with iodine to destroy the tyrosines, then it also did not bind. And the question that I had, and I spoke with Michael Pangburn this and he, basically, discounted the whole thing

because, with the comment that complement factor H is just hard to study, but the question that I had was could that data be accounted for by the polymorphism from tyrosine to histidine? I mean, could that be involved in the binding of complement factor H to the phenyl-Sepharose column? Do you remember that data? You may remember, I mean....

**Veronique Fremeaux-Bacchi:** From Michael there is no differences from the binding to factor H in \_\_\_\_\_phase and factor H in the surface. And for the apparent \_\_\_\_, there is difference with the implication in the c-terminal domain of the protein, and, at this time, I'm not\_\_\_\_\_ from the implication of SAR 7.

**Q:** Thank you. In Michael Dean's talk, he postulated that the Y402H mutation may be involved in interaction with a pathogen. So, has anyone looked or are you looking to see whether that mutation or if the CFH mutation Y402H is interacting with the pathogens that you showed on that slide?

**Veronique Fremeaux-Bacchi:** No.