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Review

Host recognition and target differentiation by factor H, a regulator of the alternative pathway of complement

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Abstract

Factor H is responsible for recognition of host cells and tissues and mediates discrimination among microbial pathogens during activation of the alternative pathway of complement (AP). Its unique structure of 20 SCR domains arranged in a flexible chain permits a variety of functional sites to interact with complement proteins and surface markers in a biological example of single-molecule combinatorial chemistry. In addition to the complement regulatory site located in the N-terminal four SCR domains, two other sites bind complement protein C3b and three sites appear to recognize a variety of polyanions that serve as host markers. Recent studies indicate that cooperativity among several C3b- and polyanion-binding sites influences the biological functions of factor H and that the degree of influence of each site varies on different cells. The engagement of one or more of the host marker recognition sites enables factor H to control activation of the AP. The absence of host-like markers allows AP activation, but many common pathogens have developed receptors for factor H or mimics of host markers of varying degrees of authenticity allowing them to escape detection by this innate defense system. Organisms using one or more of these evasive techniques include *Neisseria gonorrhoeae*, *Streptococcus pyogenes*, *Yersinia enterocolitica*, *Trypanosoma cruzi*, and the HIV virus.

Keywords: Factor H; Alternative complement pathway; Innate immunity; SCR domains; Complement regulatory proteins

1. Introduction

The alternative pathway of complement (AP) is comprised of six plasma proteins (C3, and factors B, D, H, I and P). The AP expresses an innate ability to distinguish host cells and tissues from most foreign organisms (Joiner, 1988; Pangburn, 1988; Pangburn and Müller-Eberhard, 1984). The pathway may be activated by bacteria, fungi, viruses, virus-infected cells, tumor cells and parasites. All three pathways

Abbreviations: AP, the alternative pathway of complement activation; SCR, short consensus repeats which are domains approx. 60 amino acids in length found in many complement control proteins; C3b, product of proteolytic activation of complement protein C3; CRP, C-reactive protein; E_HC3b, human erythrocytes bearing complement protein C3b on their surface; E_SC3b, sheep erythrocytes with C3b; E_RC3b, rabbit erythrocytes with C3b; ZymC3b, zymosan (yeast cell walls) with C3b; DAF, decay-accelerating factor; CR1, complement receptor number one; MCP, membrane cofactor protein

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of complement (the classical, alternative and lectin pathways) are involved in innate defense, but the AP provides the host with the broadest specificity against infectious agents and it is effective through adulthood. It is capable of rapid (5 to 20 min) activation, is extremely sensitive (full activation is elicited by a single microorganism) and is truly an innate defense system in that it does not depend on prior immunization or antibody production.

An idealistic description of target recognition by the AP is that it recognizes (and spares) the host and attacks everything else. This design is sophisticated in that it can adapt to new organisms as they arise. In reality pathogenic microorganisms employ evasion strategies that involve host mimicry (reviewed in Wurzner, 1999; Cooper, 1991; Horstmann, 1992; Joiner, 1988). Factor H with its multiple binding sites is the key recognition protein in this system. Discrimination among pathogens is due to recognition by factor H of host-like features on microorganisms which creates a spectrum of different activation rates and activation intensities among different organisms (Kozel et al., 1992; Schreiber et al., 1980; Young and Kozel, 1993).

A central question in innate immunity is how do its various systems distinguish between potential targets and the host. The molecular mechanisms by which the molecules of these systems recognize and elicit responses or prevent (i.e., to the host) responses has recently been recognized to be fundamental to understanding the adaptive immune systems of higher organisms (Carroll, 1998; Fearon, 1997; Fearon and Locksley, 1996; Hoffmann et al., 1999). Complement is the major noncellular system of innate immunity in humans and appears to have evolved in nonvertebrates long before the arrival of rearranging genes (Carroll, 1998; Hoffmann et al., 1999; Smith et al., 1999). The complement system uses a complex set of control proteins to select its targets and regulate amplification of bound C3 fragments. The cell-bound regulators (DAF, CR1, CD59, and MCP) protect host cells from cytolysis by inadvertent or misdirected complement activation (Liszewski et al., 1996). Biological particles including many host tissues lacking these membrane regulators may or may not be activators of the AP depending on the type and density of markers on their surface recognized by factor H.

2. Structure

Factor H is composed of 20 homologous short consensus repeat (SCR) domains each containing approximately 60 amino acids. Unlike many other SCR-containing proteins factor H has no other types of domains. The complete sequences of both human and mouse factor H were published more than a decade ago (Kristensen and Tack, 1986; Ripoche et al., 1988). Human factor H is heavily glycosylated and has a high sialic acid content although these can be removed without apparent affects to its function (Jouvin et al., 1984). As expected, factor H behaves as an extended molecule in solution. Its molecular weight derived from sequence (1213 amino acids + carbohydrate) and estimated from SDS gels both suggest a molecular weight of approximately 155,000 Da. Gel filtration chromatography using globular protein standards indicate a value of 300,000 Da. This has led to the proposal that it exists as a dimer in solution (Fischetti, 1991; Perkins et al., 1991). In the analytical ultracentrifuge, however, its apparent molecular weight corresponded to that of the monomer (Sim and Discipio, 1982). In the electron microscope molecules of factor H appear to have the structure of flexible 'beads-on-a-string' laid down in multiple arrangements (Discipio, 1992; Moore et al., 1989; Sim and Perkins, 1989; Weisman et al., 1990). This elongated structure seems to account for the large apparent size determined by gel filtration.

Numerous functional sites (Fig. 1) have been identified along the 20 SCR domain structure of factor H (Alsenz et al., 1984, 1985; Blackmore and Gordon, 1996; Gordon et al., 1995; Kühn et al., 1995; Pangburn et al., 1991; Ram et al., 1998a; Sharma and Pangburn, 1996). Site-directed mutagenesis has lead to the identification of three sites which bind C3b (Sharma and Pangburn, 1996) and these interact with unique sites on the C3b molecule (Alsenz et al., 1985; Jokiranta et al., 1998; Lambris et al., 1988; Sharma and Pangburn, 1996). The C3b-binding site located in the N-terminal four SCR domains possesses decay-accelerating activity for the AP C3/C5 convertase and also serves as a cofactor site for factor I, a serine protease that inactivates C3b (Alsenz et al., 1984; Kühn et al., 1995). The other two C3b binding sites shown on Fig. 1 have no direct complement regulatory activity. The other sites

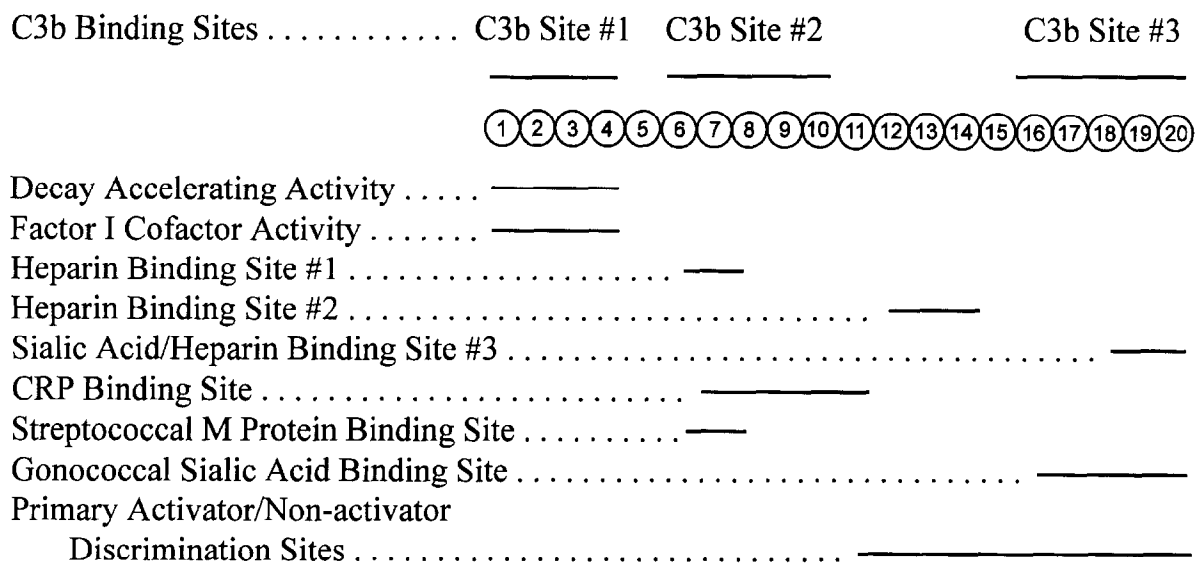

Fig. 1. Diagram of the currently known functional sites of human factor H and their locations. Factor H is composed of 20 SCR domains each composed of approximately 60 amino acids with two to eight intervening amino acids. Three sites interacting with C3b are known one of which (C3b Site #1) also expresses complement regulatory C3/C5 convertase decay-accelerating activity and factor I cofactor activity. Three polyanion binding sites have been identified and one (the site located in SCR 19–20) has been shown to specifically interact with sialic acids although all three sites bind to heparin. A number of sites are used by microorganisms to hold factor H at the cell surface and provide protection from complement activation. The C-terminal half of the protein appears to contain the primary host recognition sites and deletion of this region produces a protein with little or no ability to distinguish between human and microbial particles.

interact with polyanions of various types. The first of these is located in SCR 7 (Blackmore and Gordon, 1996), the second near SCR domain 13 (Pangburn et al., 1991) and the last in SCR domains 18–20 (Blackmore et al., 1998b; Ram et al., 1998a). The later site is the only one to have been specifically demonstrated to interact with sialic acids although all of the sites may have affinity for this marker.

Factor H is the main representative of a family of structurally or immunologically related proteins (reviewed by Zipfel et al., 1999a). The FHL-1 (factor H-like protein 1) arises by alternative splicing from factor H RNA and contains the N-terminal seven SCRs of factor H plus a small unique C-terminal. This plasma protein has all of the complement regulatory domains of factor H as well as the polyanion binding site in domain 7 and the binding site for interaction with streptococcal M protein. The FHR (factor H-related) proteins exhibit sequence similarities to factor H, but are derived from different genes (Zipfel et al., 1999a; Zipfel and Skerka, 1994). Their functions are as yet unclear, but they appear to be associated with lipoprotein complexes in human plasma (Skerka et al., 1997).

3. Regulation of AP activation

AP activation is spontaneous and involves a positive feedback, self-amplifying process which is strictly regulated in plasma by factor H. Factor H, at a concentration of 500 μg/ml, is one of the major complement proteins in plasma, second only to C3 at 1200 μg/ml. Its regulatory role in complement was first recognized in 1976 (Weiler et al., 1976; Whaley and Ruddy, 1976). In 1977 Fearon and Austen made the critical observation that C3b on the surface of activators was protected from inactivation by factors H and I (Fearon and Austen, 1977a,b). Later it was shown that the 10-fold higher affinity for C3b seen on nonactivators required the presence of sialic acid clusters or other polyanions on the surface (Fearon, 1978; Pangburn and Müller-Eberhard, 1978). On

surfaces bearing polyanions factor H binds with higher affinity to C3b and competes with the binding of factor B (Pangburn and Müller-Eberhard, 1978; Weiler et al., 1976; Whaley and Ruddy, 1976). If the C3 convertase (C3b,Bb) has already formed, factor H accelerates dissociation of Bb which loses its catalytic activity (Pangburn and Müller-Eberhard, 1986; Weiler et al., 1976; Whaley and Ruddy, 1976). On host cells DAF, a protein composed of four SCR domains, can also accelerate convertase decay (Nicholson-Weller et al., 1982). During inflammatory episodes the acute phase protein C-reactive protein (CRP) binds to factor H and to cells and may help regulate complement activation on host cells and tissues (Jarva et al., 1999; Mold et al., 1999). Inactivation of C3b by the protease factor I requires that C3b be in complex with factor H (Pangburn et al., 1977) or with a membrane-bound cofactor such as CR1 (Fearon, 1979) or MCP (Lublin and Atkinson, 1990; Seya et al., 1986). The resulting iC3b cannot form a C3 or C5 convertase. Thus, host cells are well protected if they possess membrane-bound regulatory proteins and polyanion markers that facilitate binding of factor H (Meri and Pangburn, 1990). On microorganisms lacking these regulatory elements amplification proceeds and the C3/C5 convertases formed are stabilized by binding of properdin. The potential pathogen is rapidly covered with C3b, iC3b and C3d which are ligands for receptors on phagocytic cells.

Cells bearing polyanions and other markers are protected from AP activation. Recognition of surface polyanions is not the function of bound C3b, but a function of factor H (Meri and Pangburn, 1990). While it is currently popular to think that membrane-bound regulators are the primary protectors (Atkinson et al., 1991), their absence does not result in immediate AP activation. In PNH, erythrocytes lacking these regulators circulate in plasma more than six days acquiring few C3b, but eventually lysis occurs due to accumulated C5–9. Human erythrocytes lacking both regulators *and* polyanions lyse in minutes with hundreds of thousands of C3b/cell (Pangburn, unpublished observations). Similarly, removal of sialic acid from the prototype nonactivator, sheep erythrocytes, causes them to activate the human AP (Fearon, 1978; Pangburn and Müller-Eberhard, 1978). In order to evade AP activation many pathogens express a variety of polyanions on their surfaces (Harrison and Lachmann, 1986; Jarvis and Vedros, 1987; Stevens et al., 1978). Clearly membrane-bound regulatory proteins and polyanionic markers recognized by factor H provide essential redundant systems for regulating AP activation in the host.

4. Target discrimination by factor H

The AP of complement activates spontaneously. It deposits C3b continuously on all surfaces in contact with blood (reviewed in Pangburn, 1998). Factor H in plasma, along with DAF, MCP and CR1 on host cells, controls whether the initial C3b is amplified or rapidly inactivated. Most microorganisms are strong activators of the human AP, but human pathogens activate poorly allowing only limited C3b deposition. Many of these pathogens have acquired mimics of host markers and use the same system of recognition sites on factor H as the host. Some have developed new ways to induce factor H to control activation at their surfaces. Site-directed mutagenesis of factor H has been used to try to identify sites on factor H and to understand how markers recognized by these sites affect control of the AP amplification process. These studies have led to better understanding of how both activators and nonactivators of the AP interact with factor H.

Deletion mutagenesis of factor H (Sharma and Pangburn, 1996) demonstrated that affinity for C3b-coated surfaces depended on many SCR domains located throughout the protein, not just the first four SCR at the N-terminal end where control functions reside (Fig. 1). More recently, comparison (Pangburn et al., 2000) of the roles of each area of factor H on four different biological particles has revealed that each surface utilizes a different pattern of sites on factor H and that each function of factor H depends on a unique set of sites. For example, decay acceleration of C3b,Bb on the AP nonactivators E_S and E_H was strongly dependent on both the SCR 11–15 and the 16–20 regions of factor H. Removal of both these regions caused loss of 97% of the activity expressed by SCR 1–4. On the AP activator E_R,

deletion of SCR 11–20 caused only a 4-fold loss in specific activity while on zymosan this recombinant protein had the same specific decay activity as full length recombinant factor H which was the same as normal human factor H on this surface. Further evidence that factor H recognizes differences even among strong activators was found by deletion of the SCR 6–10 region. This deletion resulted in a 75% loss of decay activity for E_RC3b,Bb, but had no effect on ZymC3b,Bb decay. Affinity and decay acceleration relied on different groups of sites on factor H and as with decay acceleration the patterns of sites regulating affinity were different on different cells. Although affinity is not a function by itself, the functional effect of high affinity would be that factor H waits longer for factor I and prevents binding of factor B for a longer period of time. Binding affinity relied heavily on SCR 6–10 on all cells. On ZymC3b deletion of this region (containing C3b site 2, see Fig. 1) caused a 97% loss of binding affinity, but loss of SCR 16–20 had no effect. In contrast deletion of SCR 16–20 caused a 50-fold decrease in binding affinity to E_SC3b but only a 5-fold change in binding to E_HC3b (Pangburn et al., 2000).

These findings suggest that the multiple C3b and polyanion sites arrayed along the flexible structure of factor H form a sophisticated recognition system. This flexibility and the length of factor H allow the protein to use its 20 domains to search for and interact with many ligands on a given target. The flexibility also frees the system from the requirement that the markers be precisely arranged on that surface. Finally, if each SCR domain of factor H contributes to the recognition pattern, and these sites work cooperatively in groups of twos, threes, fours, etc. then by simple combinatorial math factor H would have the ability to discriminate among over 10^6 target surfaces. It could be argued that the primary evolutionary force driving the development of multiple specificities in factor H was the need to recognize the increasingly diverse cells and tissues that humans now possess, but as microorganisms acquired mimics of these, perhaps the need to differentiate them from the host led to greater and greater complexity. However factor H acquired its discriminatory ability, the evidence suggests that this single protein controls host/target recognition in this ancient system of innate immunity.

5. Failures of this innate defense system

The methods pathogens use to defeat the AP of complement vary widely (reviewed in Wurzner, 1999; Cooper, 1991; Horstmann, 1992; Joiner, 1988). Those that involve factor H include polyanion mimics and expression of receptors that recruit factor H to the microbial surface. The M protein of group A streptococci binds factor H and this interaction involves domains in the SCR 6–10 region, probably SCR 7 (Blackmore et al., 1998a; Fischetti et al., 1995; Horstmann et al., 1988; Kotarsky et al., 1998; Sharma and Pangburn, 1997). The presence of factor H results in reduced deposition of C3b on the surface, greater virulence and protection from phagocytosis (Fischetti et al., 1995; Horstmann et al., 1988; Kotarsky et al., 1998). HIV viral particles capture factor H from serum through binding sites on gp41 and gp120 (Pinter et al., 1995; Stoiber et al., 1996). *Neisseria gonorrhoeae* bind factor H through a high affinity interaction with surface sialic acid structures (Jarvis and Vedros, 1987; Ram et al., 1998a; Smith et al., 1992; Wetzler et al., 1992). The binding site on factor H was demonstrated to reside in SCR 16–20, probably SCR 19–20 (Blackmore et al., 1998b; Ram et al., 1998a). Evidence for a second mechanism for binding factor H to *N. gonorrhoeae,* independent of sialic acid, has also been found (Ram et al., 1998b). Most clinical isolates of this organism express the surface protein Por1A and this protein specifically binds human factor H. YadA, a protein of the outer membrane of wild type *Yersinia enterocolitica*, binds factor H and its presence reduces C3b deposition (China et al., 1993). Sialylation of surface polysaccharides has also been demonstrated to be a virulence factor for group B streptococci (Wessels et al., 1989), *Trypanosoma cruzi* (Tomlinson et al., 1994) and *Escherichia coli* with K-1 capsular serotype (Stevens et al., 1978). Viruses that bud from host cells may pick up membrane constituents carrying protective sialic acid as in the case of Sindbis virus (Hirsch et al., 1983). These adaptations protect these organisms from aggressive AP activation and generally increase virulence.

Failures of AP recognition also include pathogenic sequelae resulting from AP activation on host cells and tissues. Membranoproliferative glomerulonephtitis type II involves activation of the AP at the

surface of the basement membrane which apparently lacks markers to recruit factor H (reviewed in West and McAdams, 1999; Zipfel et al., 1999b)). A similar pathology accompanies a deficiency of factor H in both humans (Levy et al., 1986) and a deficient strain of pigs (Hogasen et al., 1995). Inherited hemolytic uremic syndrome (HUS) is strongly, probably causally, associated with functional factor H deficiency (Pichette et al., 1994; Ying et al., 1999; Zipfel et al., 1999b). Lymphoid cells bearing CR2 (CD21) specifically activate the AP, although it is not yet clear whether this is an aid to antigen processing (Carroll, 1998; Johnson et al., 1999; Mold et al., 1988) or serves some other purpose.

6. Future directions

We are just beginning to understand the functional sites of factor H and their specificities. Understanding these specificities may allow (1) better design of biomaterials to make them more host-like (Mollnes et al., 1995), (2) the design of vaccines which promote AP activation, enhance C3b/iC3b/C3d deposition and stimulate the adaptive immune system (Fearon and Locksley, 1996), (3) development of antibiotics that block synthesis of protective microbial markers (Cooper, 1991; Horstmann, 1992; Joiner, 1988), and (4) development of means to control inadvertent or misdirected complement activation in the host (Liszewski et al., 1996). A thorough knowledge of the host recognition sites, their ligands on host cells and tissues and their functions in regulating complement activation is needed to pursue these goals.

Although there are many reports of receptors for factor H on human cells (Avery and Gordon, 1993; Dawes, 1993; Discipio et al., 1998; Erdei and Sim, 1987; Lambris and Ross, 1982), such a receptor has never been thoroughly characterized. In light of the frequency of use of factor H receptors by pathogenic organisms, it would be surprising if human cells did not utilize this abundant regulator (1000 times the concentration of DAF in blood) to provide protection for the host. New methods for receptor identification using high throughput screening or genomics should be applied to this problem.

The AP of the rabbit uses all the same components as the human system, but rabbit cells and tissues are excellent activators of the human AP. Clearly a different recognition system is used by the rabbit. Early attempts including the purification and analysis of most of the rabbit AP components failed to clarify the markers used for host protection in the rabbit (Horstmann et al., 1985). A reinvestigation of the rabbit AP perhaps aided by production of factor H chimeras would most certainly be informative.

The number of small factor H-like proteins suggests that these proteins have biological roles, perhaps in complement, perhaps in other systems. Approaches to understanding these roles could include development of ELISA or PCR-based rapid screening assays to determine population distributions and to look for natural deficiencies. The demonstration of their existence in mice would provide an experimental animal model and eventually knockout animals could be produced.

Although few reports have described a significant role for complement in the defense against cancer, factor H has been found to be a highly sensitive marker for bladder cancer (Ellis et al., 1997; Kinders et al., 1998). In culture many cancer cells synthesize significant amounts of factor H. Bladder cancers release this product into the urine and a diagnostic test using monoclonal antibodies against factor H is currently in use to detect this antigen. Although upregulation of factor H may be coincidental in cancers, it may also have functions advantageous to tumors and this should be examined more thoroughly.

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