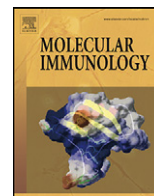




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Review

Complement evasion strategies of pathogens—Acquisition of inhibitors and beyond

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ABSTRACT

Activation of the complement system and resulting opsonisation with C3b are key events of the innate immune defense against infections. However, a wide variety of bacterial pathogens subvert complement attack by binding host complement inhibitors such as C4b-binding protein, factor H and vitronectin, which results in diminished opsonophagocytosis and killing of bacteria by lysis. Another widely used strategy is production of proteases, which can effectively degrade crucial complement components. Furthermore, bacterial pathogens such as *Moraxella catarrhalis* and *Staphylococcus aureus* capture and incapacitate the key complement component C3. The current review describes examples of these three strategies. Targeting binding sites for complement inhibitors on bacterial surfaces and complement-degrading proteases with vaccine-induced antibodies may be used to enhance a common vaccine design strategy that depends on the generation of complement-dependent bactericidal and opsonophagocytic antibody activities.

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1. Introduction

The complement system is a crucial part of the innate immunity, which has killing of pathogenic microorganisms as one of the main goals. Therefore, the ability of avoiding or preventing killing by complement is an important determinant of microbial pathogenicity. There has been a rapid expansion in our knowledge of complement evasion strategies by microorganisms over the past decade. Bactericidal activity of normal human serum (NHS) is mediated by the complement system, which can be activated through three different routes, the classical, the lectin and the alternative pathways that are triggered by various initiating proteins

that recognize bacterial ligands. Each of these pathways leads to the activation of C3 that results in deposition of opsonins C3b and iC3b on microbial surfaces. Phagocytes recognize C3b and iC3b with help of specific receptors—complement receptor 1 (CD35) and complement receptors 3 and 4 (CD11b/18 and CD11c/18), respectively. Complement activation results also in a release of anaphylatoxins C5a and C3a, which recruit and activate phagocytes. Assembly of pore-forming membrane attack complex (MAC) is important for protection against Gram-negative bacteria such as *Neisseria*. Complement is also involved in development of antibody responses by B cells and regulation of adaptive immunity (Carroll, 2008).

An area of research that has received considerable attention is the ability of pathogens to bind to complement inhibitors and evade either direct lysis (as may occur with Gram-negative bacteria) or opsonophagocytic killing (in the case of Gram-positive bacteria). Efficient complement deposition on most pathogens requires initiation of complement activation by the classical pathway. In order to inhibit classical and lectin pathways many microbes have developed the ability to bind to host C4b-binding protein (C4BP), which is a key fluid-phase inhibitor of these pathways. Protection from the alternative pathway is provided due to capturing of the major inhibitor of this pathway—factor H (FH) and its related proteins while MAC may be inhibited by vitronectin. The number of pathogens (bacteria, yeast, parasites, viruses) that are able to bind or produce complement inhibitors (Mark et al., 2007) is increasing and it can be speculated that most pathogens that must at some

Abbreviations: C4BP, C4b-binding protein; CCP, complement control protein (domain); CRASP, complement regulatory acquiring surface protein; ECM, extracellular matrix; Ecp, extracellular complement-binding protein; Efb, extracellular fibrinogen binding protein; Fba, fibrinogen binding protein; FH, factor H; FHL-1, factor H-like protein 1; FI, factor I; CFHR-1, complement factor H related protein 1; Hib, *Haemophilus influenzae* type b; Hsf, *Haemophilus* surface fibril; mAb, monoclonal antibody; LOS, lipooligosaccharide; MAC, membrane attack complex; NHS, normal human serum; NTHi, non-typeable *H. influenzae*; PAI-1, plasminogen activator inhibitor-1; por, porin; OmpA, outer membrane protein A; OMV, outer membrane vesicles; Sbi, *S. aureus* binder of immunoglobulin; SCIN, staphylococcal inhibitor of complement; Usp, ubiquitous surface protein.

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Table 1
Examples of pathogens, which bind complement inhibitors C4BP, FH and vitronectin.

Pathogen	Disease	Surface ligand	Complement inhibitor bound
<i>Neisseria gonorrhoeae</i>	Gonorrhea, disseminated gonococcal infection	Porin 1A (loop 1) Porin 1B (loops 5, 6) Type IV pili (pilC)	C4BP CCP1 C4BP CCP1 C4BP CCP1-2
<i>Neisseria meningitides</i>	Meningitis, sepsis	Porin A OpaA: outer membrane protein A	C4BP CCP2-3 Vitronectin
<i>Streptococcus pyogenes</i>	Pharyngitis, necrotizing fasciitis, rheumatic fever	M proteins (hypervariable region) Fba: fibrinogen binding protein	C4BP CCP1-2 FH, FHL-1 CCP7 FH, FHL-1 CCP7
<i>Escherichia coli</i> K1	Neonatal meningitis	OmpA: Outer membrane protein A (N-terminus)	C4BP mainly CCP3, CCP8
<i>Haemophilus influenzae</i>	Otitis media, sinusitis, bronchitis	? ? Hsf: <i>Haemophilus</i> surface fibril	C4BP CCP2, CCP7 FH CCP 6-7, CCP18-20 Vitronectin, N-terminus
<i>Moraxella catarrhalis</i>	Otitis media, sinusitis, bronchitis	Usp1, 2: Ubiquitous surface protein 1 and 2 UspA2	C4BP CCP2, CCP7 Vitronectin
<i>Borrelia recurrentis</i> and <i>duttonii</i>	Relapsing fever	? ?	C4BP FH
<i>Borrelia burgdorferi</i>	Lyme disease	BbCRASP-1 BbCRASP-2 BbCRASP-3/4/5	FH, FHL-1 CCP5-7, CCP19-20 FH, FHL-1 CCP5-7, CCP19-20 FH, CCP19-20, CFHR-1
<i>Candida albicans</i>	Candidiasis in immuno-compromised, sepsis	? Gpm1p Integrin-like	C4BP CCP1-2, CCP6 FH, FHL-1 CCP6-7, 19-20 Vitronectin
<i>Aspergillus</i> spp.	Systemic infections in immunocompromised	? ?	C4BP FH, FHL-1 CCP1-7, CCP20, CFHR1

stage survive contact with mucosal surfaces and blood are able to protect themselves by this mechanism (Table 1). Complement is particularly important during the early stage of infection and it is amply available not only in blood but also on mucosal surfaces such as bronchial, cervical and intestinal epithelial surfaces. Another bacterial complement evasion strategy that has been defined recent years is to capture and inactivate the central molecule of all pathways of complement, C3. Furthermore, proteases with specificity for complement factors can efficiently destroy bactericidal activity of serum. This review is focused on these three different strategies employed by bacteria to survive attack from the complement system (Fig. 1).

2. Complement inhibitors

2.1. C4BP

C4BP inhibits both the classical and lectin pathways of complement by acting as a cofactor for factor I (FI) mediated degradation of C4b and it also accelerates the decay of the classical pathway C3 convertase (Blom et al., 2004). In addition C4BP contributes as a FI cofactor to the cleavage of C3b and may down-regulate the alternative pathway (Blom et al., 2003). C4BP is a large plasma protein consisting of seven identical α -chains and a unique β -chain, which are covalently linked together (Kask et al., 2002). The α - and β -chains contain eight and three complement control protein (CCP) domains, respectively (Fig. 2). CCP domains consist of approximately 60 amino acids that form a compact hydrophobic core surrounded by five or more β -strands organized into β -sheets and are typical components of complement inhibitors. C4BP appears as a spider-like structure by electron microscopy with tentacles protruding from the central core (Dahlbäck et al., 1983). Full C4BP deficiency has not been reported in humans while the p.Arg240His polymorphism has been found in atypical hemolytic uremic syndrome (aHUS) patients at a higher frequency than in a healthy population (Blom et al., 2008). Three isoforms of C4BP with different subunit compositions have been identified in human plasma and the major isoform is comprised of seven α -chains and one β -chain ($\alpha_7\beta_1$) (Hillarp et al., 1989). The β -chain always carries anticoagulant, vitamin K dependent protein S (Hillarp and Dahlbäck, 1988). C4BP is an acute phase protein, and its normal levels of around 220 $\mu\text{g/ml}$ can be up-regulated around 4-fold (Barnum and Dahlbäck, 1990).

2.2. Factor H

The alternative pathway is regulated by FH via binding of C3b, inhibiting the formation and accelerating the decay of the alternative pathway C3-convertase and acting as a cofactor for the FI-mediated cleavage of C3b (Rodriguez de Cordoba et al., 2004). FH is a 150 kDa glycoprotein found in human plasma, composed of 20 CCP domains (Ripoche et al., 1988) and inhibits complement both in fluid phase and on cell surfaces. C3b is inactivated by FH in the fluid phase, whereas inactivation of surface bound C3b by FH is depen-

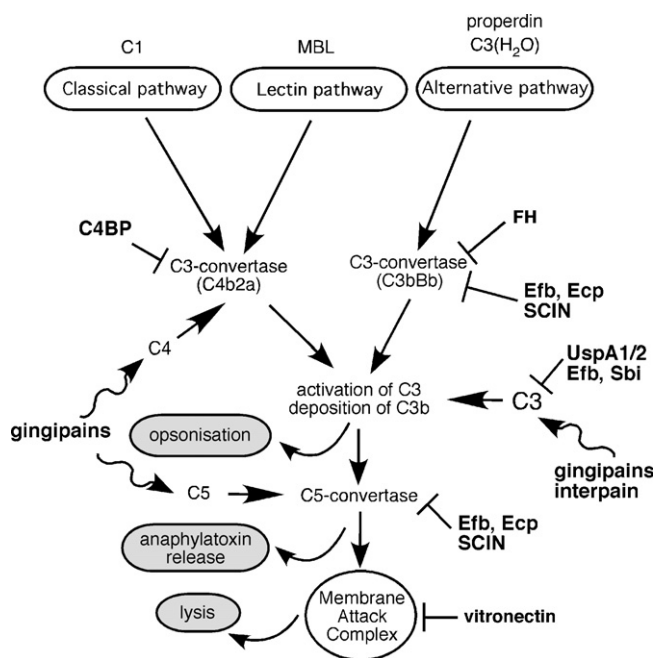


Fig. 1. The scheme of complement with indicated sites of action of bacterial proteins. Three pathways by which the human complement system can be activated and their physiological effects: opsonisation of pathogens and immune complexes for phagocytosis, release of anaphylatoxins and lysis. Furthermore, sites of action of host (C4BP, FH, vitronectin) and bacterial complement inhibitors are indicated.

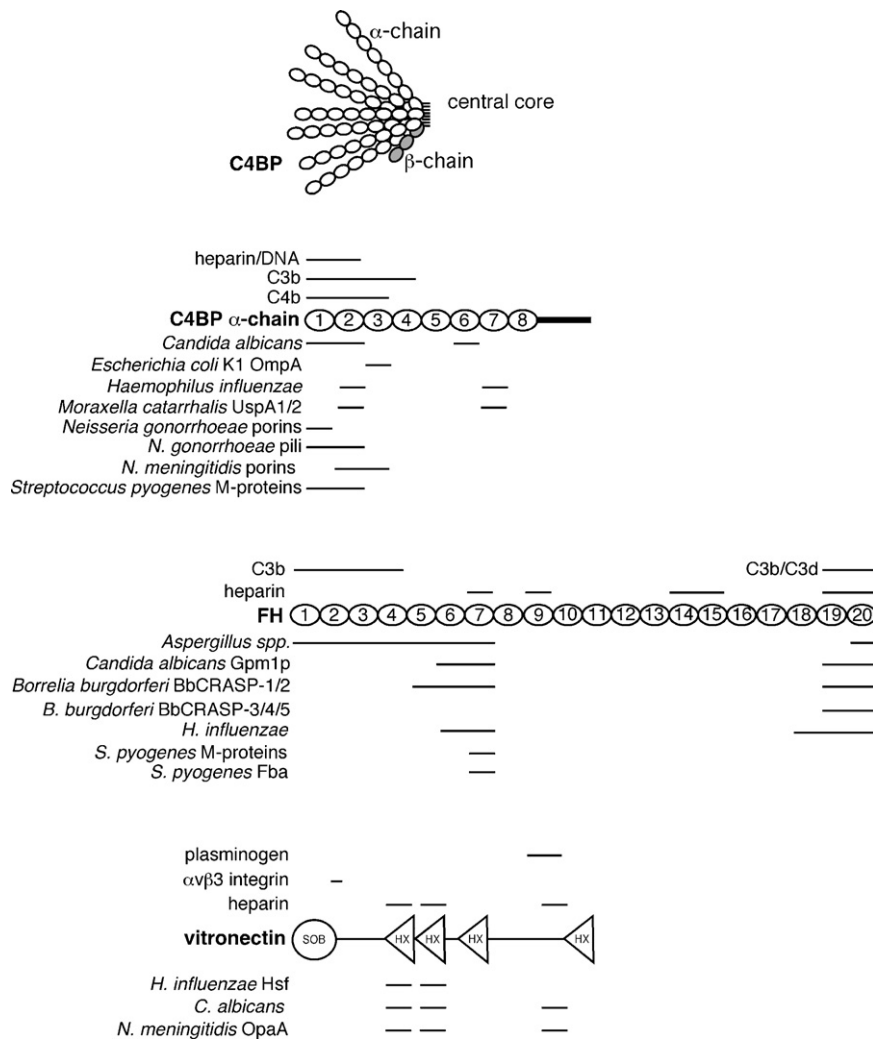


Fig. 2. C4BP, FH and vitronectin with indicated binding sites for bacterial ligands. Major form of C4BP is composed of seven identical α -chains and one β -chain held together by disulphide bridges and hydrophobic interactions in the central core. FH is a single chain protein composed of 20 CCP domains. Vitronectin has the somatomedin B domain (SOB) at its N-terminus followed by four hemopexin (HX) domains. These complement inhibitors interact with a number of bacterial ligands but also with endogenous proteins such as C4b, C3b, heparin and DNA.

dent on the chemical composition of the surface to which C3b is bound. Furthermore, FH has an important role in the discrimination between self (non-activating) and non-self (activating) surfaces. FH has high affinity for C3b when the molecule is deposited on human cells (non-activators), which are coated with sialic acid and glycosaminoglycans (Meri and Pangburn, 1990). This allows the control of the alternative pathway activation on self-surfaces. Several human diseases, including aHUS, age-related macular degeneration, and membranoproliferative glomerulonephritis type II, involve mutations and polymorphism of the FH gene (Jozsi and Zipfel, 2008).

In addition to FH, the FH family consists of six multifunctional serum proteins (Zipfel et al., 2002). Factor H-like protein 1 (FHL-1) and five Factor H-related proteins (CFHR1–5) are additional members of this group. FHL-1 is also a regulator of the alternative pathway as it displays cofactor and decay-accelerating activity and it is a product of alternative splicing of the FH gene (Estaller et al., 1991).

2.3. Vitronectin

The multifunctional glycoprotein vitronectin that is found both in plasma and in the extracellular matrix (ECM) plays important

roles in hemostasis, cellular adhesion, and in the regulation of the terminal pathway of complement and MAC (Schvartz et al., 1999). Vitronectin exists as a 75-kDa protein in the ECM and as two truncated forms in plasma. These two forms, 65 and 10 kDa, are held together by a disulfide bond. The N-terminal part of vitronectin is equivalent with somatomedin B and binds plasminogen activator inhibitor-1 (PAI-1). This domain is followed by an Arg-Gly-Asp binding sequence that is important for the vitronectin-dependent movement and attachment of epithelial cells by interacting with several integrins including the $\alpha\beta 3$ integrin. Vitronectin also has three heparan sulphate-binding domains and binds plasminogen at its C-terminus. The importance for vitronectin-mediated interactions with bacteria was recently shown with pneumococci that utilize vitronectin and its integrin binding capacity for adhesion and invasion of epithelial cells (Bergmann et al., 2009). Another example is that bacterial lipopeptides can be sensed via vitronectin by a Toll-like receptor 2-integrin $\beta 3$ complex (Gerold et al., 2008).

The formation of the lytic pore, i.e., MAC, occurs by transition of hydrophilic complement components C5b, C6, C7, and C8 resulting in polymerization of C9. Vitronectin inactivates the MAC by inhibiting the C5b–7 complex at its membrane-binding site. The insertion of the complex into the cell membrane is hereby inhibited preventing lysis of the microbe (Preissner, 1991). Interestingly, this complex

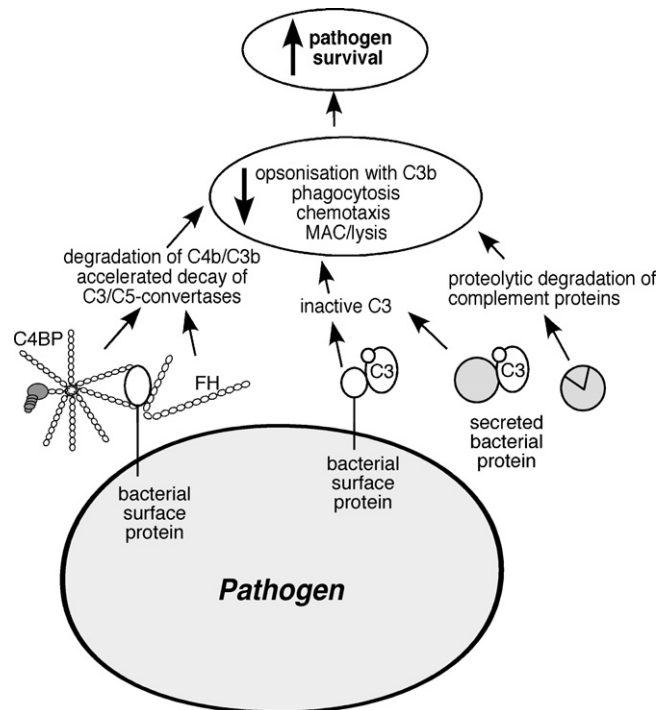


Fig. 3. Examples of strategies employed by pathogens for protection from complement. C4BP and FH bound to the surface of a pathogen inhibit C3/C5-convertases and serve as FI cofactors in degradation of C3b and C4b. C3 can be captured by bacterial proteins in a way that does not allow its participation in the complement cascade. Furthermore, complement proteins can be degraded by bacterial proteases. All these phenomena lead to decrease in opsonisation with C3b and impaired chemotaxis and therefore less efficient phagocytosis. Furthermore, assembly of MAC and lysis are also inhibited.

is still able to bind C8 and C9 to form soluble C5b-8 and C5b-9 complexes, but the latter is non-lytic. It has also been shown that vitronectin in solution directly inhibits the C9 polymerization, and hence indirectly can block the pore formation. Bacteria do not only use vitronectin as a bridge for adhesion but also bind vitronectin in order to prevent the formation of the MAC and hereby significantly increase survival in serum.

3. Examples of binding of complement inhibitors to microbial pathogens

3.1. *Streptococcus pyogenes*

Streptococcus pyogenes (group A streptococcus; GAS) is one of the most common causes of bacterial infections in humans and is associated with pharyngitis, impetigo, necrotising fasciitis, septicemia and toxic shock syndrome sometimes followed by rheumatic fever or glomerulonephritis. Important virulence factors are M proteins that have been studied extensively due to their successful ability to inhibit phagocytosis allowing bacteria to multiply in blood (Fischetti, 1989; Lancefield, 1962). A remarkable property of M proteins is their ability to bind a number of plasma proteins including C4BP (Thern et al., 1995) and FH (Horstmann et al., 1988), (Fig. 3). Studies of several different M proteins showed that the high-affinity binding site for C4BP is localized to the hypervariable N-terminal region (Johnsson et al., 1996). This finding implies that the interaction with C4BP is of physiological importance, since the ability to bind C4BP has been retained in spite of extensive sequence variation (Persson et al., 2006). M proteins interact with CCP1–CCP2 of the C4BP α -chain (Accardo et al., 1996; Blom et al., 2000) and recently, structure of complexes between C4BP and M-proteins was described (Andre et al., 2006; Jenkins et al., 2006). The interaction

with C4BP is restricted to primates (Accardo et al., 1996; Åkerström et al., 1991), a finding that may be related to the fact that *S. pyogenes* normally causes disease only in humans. Most importantly, the ability to bind C4BP was recently correlated with phagocytosis resistance of these bacteria (Carlsson et al., 2003; Morfeldt et al., 2001). It appears that deposition of complement on *S. pyogenes* occurs almost exclusively via the classical pathway, even under nonimmune conditions, but is down regulated by bacteria-bound C4BP, providing an explanation for the ability of bound C4BP to inhibit phagocytosis (Carlsson et al., 2003).

The ability of the M proteins to bind FH results in reduced activation of the alternative pathway and has been shown to contribute to the capacity of *S. pyogenes* to evade phagocytosis (Horstmann et al., 1988). However, the importance of FH-M protein binding to phagocytosis resistance has been questioned in a study using mutants of M protein lacking ability to bind FH that was still able to resist phagocytosis (Kotarsky et al., 2001). In addition to bind FH, some of the M proteins have been shown to bind FHL-1 (Kotarsky et al., 1998). The fibrinogen-binding protein (Fba) is another FH/FHL-1 binding protein that was first identified in *S. pyogenes* serotype M1 isolates but can be found in at least 18 different serotypes of *S. pyogenes* (Pandiripally et al., 2002; Terao et al., 2001). Intriguingly, the binding of Fba to FHL-1 promotes entry of *S. pyogenes* into human epithelial cells (Pandiripally et al., 2003).

3.2. *Escherichia coli*

Escherichia coli K1 responsible for meningitis in neonates also binds C4BP (Prasadarao et al., 2002). Due to the need of a certain threshold level of bacteremia for the development of meningitis, the bacteria must have a capacity to resist serum bactericidal activity. At first it was suggested that the K1 capsular polysaccharide is necessary for survival of *E. coli* in the blood (Kim et al., 1992). It was subsequently shown that outer membrane protein A (OmpA) confers serum resistance both *in vivo* and *in vitro* (Weiser and Gotschlich, 1991), which appears to be related to the fact that CCP3 of the C4BP α -chain interacts hydrophobically with the N-terminal part of OmpA (Prasadarao et al., 2002; Wooster et al., 2006). Synthetic peptides corresponding to CCP3 sequences block the binding of C4BP to OmpA and also significantly enhance the serum bactericidal activity. In addition, an antibody directed against the N-terminal part of OmpA increased bactericidal activity of NHS. Therefore, the N-terminus of OmpA could be a suitable target for the construction of an effective vaccine that would nullify the binding of C4BP in order to permit complement attack. Interestingly, the deposition of C4BP from adult serum prevented the invasion of *E. coli* into brain microvascular endothelial cells while treatment with cord serum that has lower levels of C4BP than adult serum had no effect on the invasion (Maruvada et al., 2007).

3.3. *Haemophilus influenzae*

H. influenzae is an important respiratory pathogen that can be classified according to the presence of a polysaccharide capsule (Kilian, 2003). The encapsulated strains cause invasive diseases, whereas the unencapsulated and hence non-typeable *H. influenzae* (NTHi) are mainly found in local upper and lower respiratory tract infections (Broome, 1987; St Geme, 1993). An interaction between C4BP and NTHi has been identified. The binding was mediated by CCP2 and CCP7 of C4BP α -chains. The majority of the typeable *H. influenzae* (a–f) tested did not, however, bind C4BP (Hallstrom et al., 2007). Importantly, a low C4BP-binding NTHi isolate showed an increased deposition of C3b followed by a reduced survival as compared with a high-binding strain when exposed to NHS. This is in agreement with observation that C4BP bound to the surface of *H.*

influenzae retained its cofactor activity as determined by analysis of C3b and C4b degradation.

In addition to C4BP binding, *H. influenzae* has been shown to interact with FH and FHL-1 (Hallstrom et al., 2008). The majority of tested *H. influenzae* strains bound FH, including encapsulated *H. influenzae* and NTHi. Two binding domains were identified within FH; CCPs 6–7 and the C-terminal CCPs 18–20, but the FH-ligand of *Haemophilus* has not been identified yet. FH and FHL-1 retained their co-factor activity when bound to the surface of *H. influenzae* type b (Hib) and an increased survival in NHS was observed with a high FH-binding Hib isolate as compared to a low FH-binding Hib strain. In contrast to incubation with complement active NHS, Hib had a reduced survival in a complement active FH-depleted human serum, thus demonstrating that FH mediates a protective role at the bacterial surface.

Haemophilus surface fibril (Hsf) is a large autotransporter that binds vitronectin and contributes to serum resistance when Hib is exposed to NHS (Hallstrom et al., 2006). Hsf is found in encapsulated strains and the binding to vitronectin protects the bacteria against the MAC, but is also important for bacterial adhesion to the ECM. Two separate binding domains of Hsf bind vitronectin and the interaction is inhibited by heparin. The N-terminal part of vitronectin was most likely involved in the interaction with Hsf as shown in experiments with peptides. Taken together, *H. influenzae* interferes with the classical/lectin (C4BP), alternative (FH) and terminal pathways (vitronectin), and thereby reduces the complement-mediated bactericidal activity resulting in an increased bacterial survival.

3.4. *Moraxella catarrhalis*

Moraxella catarrhalis, formerly considered to be a harmless commensal in the respiratory tract, is now acknowledged as an important mucosal pathogen. It is the third leading bacterial cause of acute otitis media in children and is also a common cause of sinusitis and lower respiratory tract infections in adults with chronic obstructive pulmonary disease (Murphy, 1996). The majority (89%) of *M. catarrhalis* isolates from patients with lower respiratory tract infections are resistant to complement-mediated killing (Hol et al., 1995). C4BP binds ionically via CCP2 and CCP7 to ubiquitous surface proteins A1 and 2 (UspA1, UspA2) of *M. catarrhalis* with UspA2 being the major binder (Nordström et al., 2004). Interestingly, UspA2 mediates serum resistance of the bacteria, which could be due, at least partially, to the binding of C4BP. *M. catarrhalis* does not only bind C4BP but also inhibits the direct formation of the MAC by UspA2-mediated binding of vitronectin (Attia et al., 2006).

3.5. *Neisseria gonorrhoeae* and meningitidis

Gonococcal strains that cause disseminated gonococcal infection usually are resistant to the bactericidal action of nonimmune NHS (O'Brien et al., 1983) while those strains that cause pelvic inflammatory disease are most commonly sensitive to killing by NHS (Rice et al., 1980). Initially all gonococci that are recovered from the human genital tract are resistant to NHS, but they may lose this property upon culture (Ward et al., 1970). The addition of CMP-sialic acid to growth media results in sialylation of lipooligosaccharide (LOS) and reversion back to a resistant phenotype. This is termed unstable serum resistance, and is usually mediated by binding of FH to sialylated organisms (Ram et al., 1998b). Because gonococci are heterogeneously sialylated *in vivo* (Apicella et al., 1990; McQuillen et al., 1999), they may require mechanisms other than LOS sialylation in order to maintain the serum resistant state *in vivo*. Binding of FH to porin is one such important serum resistance mechanism (Ram et al., 1998a) providing protection from the alternative pathway of

complement. Porin is a 34–35 kD protein comprising 8 transmembrane loops, which functions as a selective anion channel and is essential for survival of the organism (Massari et al., 2003). Porin is the most abundant gonococcal outer membrane protein, and gonococci are classified into Por1A or Por1B serotypes. In addition to interaction with FH, binding of C4BP allows *Neisseria* to down-regulate the classical and the lectin pathways. Binding of C4BP to *N. gonorrhoeae* correlates with the serum resistance as shown by the fact that 8 out of 10 tested resistant Por1A strains bound C4BP, while the only serum-sensitive Por1A strain did not (Ram et al., 2001). Furthermore, 8 out of 11 serum resistant Por1B strains bound C4BP while none of the serum-sensitive Por1B strains bound C4BP. In the subsequent studies using mutants of C4BP lacking single domains or carrying point mutations that abrogated binding to bacteria (Jarva et al., 2007) a strict correlation between binding of C4BP and serum resistance of *N. gonorrhoeae* was detected. Strains bearing hybrid Por1A/Por1B molecules showed that loop 1 of PorA1 was required, but not sufficient, for binding to CCP1 of C4BP α -chain while a region spanned by Por1B loops 5 and 7 was necessary for C4BP binding (Ram et al., 2001). LOS heptose (Hep) glycan substitutions influence gonococcal serum resistance (Ram et al., 2007), which may be in part related to the fact that the proximal glucose on Hep1 is required for C4BP binding to Por1B-bearing gonococcal strains MS11. Apart from porins, C4BP also interacts with type IV pili from *N. gonorrhoeae* (Blom et al., 2001), which are elongated structures extending from the bacterial surface and in their absence the bacteria are not able to establish infection (Kellogg et al., 1968; Swanson et al., 1987).

Interestingly, strains of *N. gonorrhoeae* that resisted killing by human serum complement were killed by serum from rodent, lagomorph, and primate species, which cannot be readily infected experimentally with this organism and whose C4BP molecules did not bind to *N. gonorrhoeae* (Ngampasutadol et al., 2005). In contrast, *Yersinia pestis*, an organism that can infect virtually all mammals, bound species-specific C4BP and uniformly resisted serum complement-mediated killing by these species. Serum resistance of gonococci was restored in these sera by addition of human C4BP. An exception was serotype Por1B-bearing gonococcal strains that previously had been used successfully in a chimpanzee model of gonorrhea that simulates human disease. Por1B gonococci bound chimpanzee C4BP and resisted killing by chimpanzee serum, providing insight into the host restriction of gonorrhea (Arko, 1989) and addressing why Por1B strains, but not Por1A strains, have been successful in experimental chimpanzee infection. Interestingly, species specificity may also be provided by FH (Ngampasutadol et al., 2008). These findings may lead to the development of better animal models for gonorrhea and also have implications in the choice of complement sources to evaluate vaccine candidates.

Neisseria meningitidis is an important cause of meningitis and sepsis. Host defense against meningococci requires complement and individuals deficient in properdin or MAC components have an increased susceptibility to recurrent neisserial infections. Binding of C4BP was tested to wild-type group B meningococcus strain and to 11 isogenic mutants thereof that differed in capsule expression, LOS sialylation, and/or expression of either PorA or PorB3. The strains lacking PorA bound significantly less C4BP while deleting PorB3 did not influence C4BP binding, and the presence of polysialic acid capsule reduced C4BP binding by 50% (Jarva et al., 2005). The C4BP-PorA interaction was ionic, suggested by the observation that optimal binding of C4BP to meningococci occurred in hypoosmolar buffers. PorA-expressing strains were also more resistant to complement lysis than PorA-negative strains in a serum bactericidal assay implying that binding of C4BP thus allows *N. meningitidis* to escape classical pathway activation.

PorB3 expressed by *N. meningitidis* is a homologous protein to the FH-binding protein Por1A of *N. gonorrhoeae* (Ram et al., 1998a;

Wolff and Stern, 1991). However, PorB3 did not show any binding of FH (Madico et al., 2006). Instead, another FH-binding protein was identified in *N. meningitidis* - the meningococcal vaccine candidate GNA1870, which is expressed by all *N. meningitidis* strains studied (Madico et al., 2006). The level of GNA1870 expressed on the surface of the bacteria correlated with the amount of FH bound to the meningococci. The GNA1870 deficient mutant showed an increased deposition of C3 and a decreased survival in NHS compared to the wild type expressing GNA1870. GNA1870 is a potential vaccine candidate because antibodies directed against it promote killing (Beernink et al., 2008) and could promote opsonophagocytosis of the bacteria by blocking the binding of FH to the pathogen. The ability of both *N. gonorrhoeae* and *N. meningitidis* to bind FH is restricted to human FH that also may explain why these species do not cause natural infections in experimental animal models (Granoff et al., 2009; Ngampasutadol et al., 2008).

Furthermore, ligand blots demonstrated that vitronectin bound specifically to the heparin-binding outer-membrane protein OpaA of *N. meningitidis*, but that coating OpaA with the sulphated polysaccharide heparin was required for the interaction to occur (Duensing and Putten, 1998). Bound vitronectin could be dissociated from OpaA-heparin-vitronectin complexes by the addition of excess heparin. Therefore, a novel mechanism was proposed according to which sulphated polysaccharides act as molecular bridges, linking the glycosaminoglycan-binding sites of vitronectin and gonococcal OpaA.

3.6. *Borrelia* species

Relapsing fever is a rapidly progressing and severe septic disease caused by *Borrelia* spirochetes. There are two forms of the disease - epidemic relapsing fever caused by *Borrelia recurrentis* and transmitted by lice, and the endemic form caused by several *Borrelia* species, such as *B. duttonii* and transmitted by soft-bodied ticks. Following vector bites, the spirochetes enter the bloodstream and persist in plasma despite the development of specific antibodies, which leads to fever relapses and high mortality. Both *B. recurrentis* and *B. duttonii* are serum resistant and acquire FH on their surfaces (Meri et al., 2006) in a similar way to that of the Lyme disease pathogen, *Borrelia burgdorferi sensu stricto* (Hellwege et al., 2001; Kraiczy et al., 2001). Furthermore, the relapsing fever spirochetes specifically bind C4BP (Meri et al., 2006) and both complement inhibitors retain their functional activities when bound to the surfaces of the spirochetes. Complement regulator-acquiring surface proteins (CRASPs) are surface exposed, functionally related proteins of *B. burgdorferi* that bind FH, FHL-1 or complement FH-related protein 1 (CFHR-1) (Alitalo et al., 2004; Haupt et al., 2007; Kraiczy et al., 2001). CRASP expression of the *Borrelia* strains correlates with serum resistance, suggesting that FH, FHL-1, and CFHR-1 attached to the surface of the pathogen regulate complement (Brooks et al., 2005; Haupt et al., 2007). CFHR-1 is acquired by serum-resistant strains but not by serum sensitive strains of *B. burgdorferi*, suggesting a role of CFHR-1 for immune evasion of the pathogen (Haupt et al., 2007). On the other hand, it has been shown that FH deficient mice can be infected by *B. burgdorferi* to an equal degree as the wild type mice (Woodman et al., 2007), which prompted the authors to suggest that the interaction with FH is not essential for a successful infection. However, FH deficient mice have very low levels of C3 and may therefore not be the most accurate model for assessment of the role of FH, which is the main regulator of C3. It is of course still possible that evasion of the complement-mediated killing of *B. burgdorferi* involves additional mechanisms to FH binding.

B. burgdorferi has several reservoirs, including birds, rodents and dogs and it has been shown that in addition to bind human FH, CRASP-3, CRASP-4 and CRASP-5 bind FH from other species (e.g. mouse, rat, rabbit, and horse) (Stevenson et al., 2002). Thus, it is

suggested that the expression of different CRASP proteins can allow the *B. burgdorferi* to resist complement-mediated killing in a wide range of potential hosts.

3.7. *Candida albicans* and *Aspergillus fumigatus*

Candida albicans is the most common human pathogenic yeast causing cutaneous and mucocutaneous candidiasis (Pfaller and Wenzel, 1992). In healthy individuals the cellular form of yeast is often present as a commensal. However, *C. albicans* can also cause life threatening systemic infections especially in immunocompromised and granulocytopenic patients (Fisher-Hoch and Hutwagner, 1995). *C. albicans* activates all three pathways of the complement, but both yeast and hyphal forms of *C. albicans* capture complement inhibitors FH and FHL-1 (Meri et al., 2002) as well as C4BP (Meri et al., 2004). In hyphae, a prominent binding site for complement inhibitors was identified at the tip, which has for a long time been considered an important structure for tissue penetration and pathogenesis. The binding is mediated by CCP1-2 of C4BP α -chain (Meri et al., 2004). The phospho-glycerate mutase Gpm1p from *C. albicans* was the first identified FH-binding protein of yeast (Poltermann et al., 2007). FH and FHL-1 were shown to be ligands for Gpm1p, whereas C4BP showed no binding. Two binding regions were identified within FH, CCPs 6-7 common to both FH and FHL-1 and CCPs 19-20 unique for FH. The Gpm1p deficient mutant showed reduced binding to FH. However, the FH binding to the mutant was not completely abolished and this result indicates that several FH binding proteins of *C. albicans* remain to be identified.

Binding of vitronectin is common in clinical isolates of *C. albicans* (Jakab et al., 1993). Vitronectin binding to *C. albicans* was significantly inhibited by heparin, suggesting an interaction of the organism with the glycosaminoglycan-binding region of vitronectin (Limper and Standing, 1994). *C. albicans* in the yeast phase expresses receptors antigenically related to the vertebrate $\alpha\beta 3$ and $\alpha\beta 5$ integrins, which mediate its adhesion to vitronectin (Spreghini et al., 1999).

Recently, binding of C4BP to a pathogenic mold (*Aspergillus* spp.) was also reported (Vogl et al., 2008). *Aspergillus fumigatus* is the major cause of fungal infections in immuno-compromised patients, including severe systemic infections (Brakhage, 2005). *A. fumigatus* conidia has been shown to bind FH, FHL-1 and CFHR1 and this may contribute to the evasion of the host complement attack (Behnsen et al., 2008). CCPs 1-7 of FH and FHL-1 and CCP 20 of FH were identified as the binding regions of *A. fumigatus* within FH/FHL-1 and bound to the surface of the conidia FH retained its regulatory activity.

4. Inhibition of C3 by *Moraxella* and *Staphylococcus aureus*

The complement protein C3 is one of the most abundant proteins in serum and a central molecule of the complement system. Upon activation, C3 undergoes conformational changes and proteolytic cleavages, which results in amplification of complement, anaphylatoxin release (C3a), cell lysis and phagocytosis (Janssen and Gros, 2007). C3 forms a link between all three pathways of complement activation and it is involved in the interactions between the innate and acquired immune systems (Toapanta and Ross, 2006). Therefore, some pathogens target and incapacitate C3. *M. catarrhalis* UspA1 and UspA2 interfere with the alternative pathway by neutralizing C3 in addition to the binding of C4BP (Nordstrom et al., 2005). *M. catarrhalis* readily absorbed C3 from NHS in which complement was inactivated by EDTA and non-covalently bound purified methylamine-treated C3 (C3met). An intriguing finding was that pre-incubation of serum with recombinant UspA1⁵⁰⁻⁷⁷⁰ and UspA2³⁰⁻⁵³⁹ resulted in absorption of C3 and increased the survival of a *M. catarrhalis* UspA1/A2 deficient mutant. Importantly,

when *M. catarrhalis* was pre-incubated with EDTA-treated NHS the bacteria was not phagocytosed by polymorphonuclear leukocytes, indicating that C3 on the bacterial surface was inactive and did not function as an opsonin.

Since *M. catarrhalis* is a respiratory pathogen, the neutralization of C3 most likely also occurs at the mucosal surface. This is supported by the fact that in disease states such as acute otitis media there is a strong ongoing complement activation (Narkio-Makela et al., 1999). Thus, *Moraxella*-dependent binding of C3 may represent an important feature of evading the mucosal defence. The *M. catarrhalis*-dependent C3 binding is, however, relatively unique since several other bacteria including *Moraxella* subspecies do not bind C3 to the bacterial surface (Nordstrom et al., 2005). Another interesting observation is that *M. catarrhalis*, like other Gram-negative bacteria releases outer membrane vesicles (OMVs), which carry some of the underlying periplasm, together with all the components of the outer membrane layer including its integral proteins. *M. catarrhalis* OMVs are equipped with UspA1 and A2, which has been demonstrated in clinical nasopharyngeal specimens from a 9-year old child (Tan et al., 2007). UspA-expressing OMVs specifically bind C3 and hence counteract the complement cascade. Most importantly, *Moraxella* OMVs also increase the survival of the relatively serum susceptible NTHi when exposed to human serum suggesting a collaboration between these two respiratory pathogens dwelling in the mucosa.

Staphylococcus aureus is a human pathogen responsible for a wide range of diseases that vary in severity. The species is the cause of skin and soft-tissue infections and more serious and potentially fatal conditions, including bacteremia, necrotizing pneumonia, and endocarditis. As with all bacteria complement plays a principal role in the immune response against *S. aureus* (Sakinienė et al., 1999). *S. aureus* expresses and secretes several proteins that interfere with the complement system and thus protect the bacteria against complement-mediated attacks. Extracellular fibrinogen binding protein (Efb) is a secreted protein that binds the C3d domain of the C3 molecule and thereby inhibits the deposition of C3b on the surface of *S. aureus* (Lee et al., 2004). The binding of C3-inhibitory domain of Efb (Efb-C) to C3d changes the overall solution conformation of C3 so it is unable to be cleaved into C3b and unable to participate in successful activation of the complement cascade (Hammel et al., 2007b). Efb is also able to bind the activated fragment C3b with high affinity and induce conformational changes. Ehp, another protein secreted by *S. aureus*, which shows 44% identity to Efb has also been shown to bind the C3d fragment of C3 and is able to inhibit C3b deposition on sensitized surfaces by the alternative pathway (Hammel et al., 2007a). Furthermore, *S. aureus* binder of immunoglobulin (Sbi) binds C3d and is able to inhibit the alternative pathway (Burman et al., 2008; Haupt et al., 2008; Upadhyay et al., 2008). Mutational analysis of Sbi showed that loss of C3d binding resulted in loss of alternative pathway inhibitory capacity further indicating the importance of the C3 binding for the pathogen (Upadhyay et al., 2008). In addition, Efb, extracellular complement-binding protein (Ecp), and staphylococcal inhibitor of complement (SCIN) are all inhibitors of the C3b containing convertases (e.g., the C3 convertase of the alternative pathway C3bBb and the C5 convertases C4b2aC3b and C3bBbC3b) (Jongerijs et al., 2007). It is suggested that the binding of these proteins to C3b in the convertases changes the conformation of C3b and this probably affects the activity of the protease complex.

5. Soluble proteases from bacterial pathogens

Pathogens target complement also by degrading crucial complement components such as C3 and C5. Thus, a number of bacterial proteases such as cysteine protease SpeB from *S. pyogenes* cleave C3 (Terao et al., 2008). The same protease also degrades properdin,

which serves as enhancer of the alternative pathway when intact (Tsao et al., 2006). *S. pyogenes* also produces C5a peptidase, which cleaves C5a near its N-terminus and thus prevents its ability to attract and activate neutrophils (Ji et al., 1996). The PgtE protease of *Salmonella enterica* degrades C3, C4 and C5 thereby protecting the bacterium during its transient extracellular phase (Ramu et al., 2007). The gelatinase of *Enterococcus faecalis* also inactivates C3 (Park et al., 2007).

Periodontitis is an inflammatory disease of the supporting structures of the teeth and is caused among others by *Porphyromonas gingivalis*. *P. gingivalis* is very resistant to killing by human complement (Okuda et al., 1986; Sundqvist and Johansson, 1982), which is present at 70% of serum concentration in gingival fluid. Cysteine proteinases of the gingipain family are implicated as the major factor exerting protection against complement. Gingipains are responsible for more than 85% of general proteolytic activity generated by this bacterium (Potempa et al., 1997). These enzymes are encoded by three strictly conserved among *P. gingivalis* strains and clinical isolates genes (*rgpA*, *rgpB*, and *kgp*) coding for two closely related arginine-Xaa specific (RgpA and RgpB) and one lysine-Xaa specific (Kgp) proteinases, which occur in multiple molecular forms due to proteolytic processing and glycosylation (Potempa et al., 2003). All three gingipains degrade multiple complement components with arginine specific gingipains (HRgpA and RgpB) being more efficient than lysine specific gingipain (Kgp) (Popadiak et al., 2007). Interestingly, low concentrations of gingipains appear to activate complement factors C3, C4 and C5 as they preferentially aim at the α -chains of these proteins (Schenkein and Berry, 1988; Wingrove et al., 1992). This may lead to release of anaphylatoxins C3a and C5a as well as activated forms: C4b, C3b and C5b. At higher concentrations the proteases simply degrade these three complement factors into small fragments so that they can no longer propagate complement cascade. For C3 this was observed *in vivo* in a guinea-pig model (Sundqvist et al., 1984). The three gingipains are also able to activate C1 complex and cause its deposition on a surface that normally does not serve as an activator (Popadiak et al., 2007). This may lead to a certain level of inflammation that provides access to nutrients for the bacteria and allows colonization. At higher concentrations of bacteria and gingipains, the complement system becomes incapacitated by multiple cleavages of several participating proteins.

Prevotella intermedia is one of the bacterial pathogens that has been implicated to cause periodontitis and is often recovered from subgingival plaque in patients suffering from acute necrotizing gingivitis, pregnancy gingivitis and chronic periodontitis (Loesche et al., 1982). Interpain A is a cysteine proteinase expressed by *P. intermedia* that appears to be similar to *S. pyogenes* SpeB (Mallorqui-Fernandez et al., 2007; Potempa et al., 2005). Interpain A is expressed by the majority of *P. intermedia* strains and can be detected in gingival crevicular fluid. Interpain A destroys efficiently bactericidal activity of human serum because it degrades C3 (Potempa et al., 2009). *P. intermedia* has been known to co-aggregate with *P. gingivalis*, and it was shown that interpain A has a synergistic effect with gingipains on complement degradation. Importantly, *Prevotella* species readily acquire resistance towards antibiotics (Walker, 1996) and deeper knowledge of how infection and serum resistance occur will be crucial for the development of alternative treatments to periodontal disease.

6. Conclusions

All bacterial pathogens have developed sophisticated strategies to conquer the immune defence systems such as the complement system and hence increase their survival. Recent years have provided a growing body of information on complement evasion strategies used by various pathogens. Modern techniques have

made it possible to study in detail different evasion mechanisms at the molecular level. During the coming years the main focus most likely will be on finding suitable vaccine candidates among bacterial proteins that interact with complement and on defining new ways to manipulate the complement system in order to develop novel therapeutic approaches utilizing the knowledge gained from the pathogens.

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