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Contribution of interactions between complement inhibitor C4b-binding protein and pathogens to their ability to establish infection with particular emphasis on *Neisseria gonorrhoeae*.

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Abbreviations: C4BP – C4b-binding protein; CCP – complement control protein (domain); DGI - disseminated gonococcal infection; FH – factor H, FI – factor I, Hep - heptose; mAb - monoclonal antibody; LOS – lipooligosaccharide; MAC – membrane attack complex; NHS – normal human serum; NTHi - non-typeable *Haemophilus influenzae*; por – porin; OmpA - outer membrane protein A; PID - pelvic inflammatory disease; Usp - ubiquitous surface protein

Abstract
Complement activation and resulting opsonisation with C3b form key arms of the innate immune defense against infections. However, a wide variety of pathogens subvert complement attack by binding host complement inhibitors, which results in diminished opsonophagocytosis and killing of bacteria by lysis. Human C4b-binding protein (C4BP) binds *Neisseria gonorrhoeae* and *Streptococcus pyogenes*, both uniquely human pathogens. This binding specificity is circumvented by other bacterial species, which bind C4BP from numerous mammalian hosts that they infect. Binding of C4BP to *Neisseria* is mediated by outer membrane proteins porins and appears to be one of the main factors mediating serum resistance. Targeting C4BP binding sites on bacterial surfaces with vaccine-induced antibodies may block binding of C4BP and enhance a common vaccine design strategy that depends on the generation of complement-dependent bactericidal and opsonophagocytic antibody activities.

1. Introduction

Over the past decade there has been a rapid expansion in our knowledge of complement evasion strategies by microorganisms. An area 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-positives). Efficient complement deposition on most pathogens requires initiation of complement activation by the classical pathway. In order to inhibit classical pathway several microbes have developed the ability to bind to host C4b-binding protein (C4BP), which is a key fluid-phase inhibitor of this pathway. In this review we provide a brief overview of the role of C4BP in microbial complement evasion strategies. Emphasis is placed on the interactions of C4BP with *Neisseria gonorrhoeae*.

Killing by normal human serum (NHS) is mediated by the complement system, which is crucial in the defense against microbial pathogens. Complement 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 (Fig. 1). Each of these pathways lead to the activation of C3 that results in deposition of the opsonin, C3b, on microbial surfaces, as well as assembly of pore-forming membrane attack complexes (MAC) that, in the example of gram-negative bacteria, directly kill the organisms. Individuals deficient in alternative or terminal complement pathway components are particularly susceptible to neisserial infections [1], emphasizing the importance of complement in the defense against pathogenic neisseriae. Complement components are present in mucosal secretions [2], therefore mucosal pathogens such as *N. gonorrhoeae* come into contact with complement already at the site of initial colonization. Complement component C3 is present in functional amounts at the cervical level [2, 3], is synthesized in the endometrial glandular epithelium [4, 5], and binds to gonococci *in vivo* [6]. While the alternative pathway is important in amplifying C3 deposited on the gonococcal surface, the classical pathway of complement is required to initiate C3b deposition and also for complement-mediating killing. A key soluble phase classical pathway inhibitor is C4BP.

2. Complement inhibitor 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 (C2aC4b) [7]. In addition, C4BP, like the major inhibitor of the alternative pathway factor H (FH), contributes as a FI cofactor to the cleavage of C3b and may down-regulate the alternative pathway [8]. C4BP is a large plasma protein consisting of seven identical α-chains and a unique β-chain, which are covalently linked together [9]. The α- and β-chains contain eight and three complement control protein (CCP) domains, respectively. 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 [10]. C4BP appears as a spider-like structure by electron microscopy with tentacles protruding from the central core [11]. Full C4BP deficiency has not been reported in humans while the p.Arg240His polymorphism has been found in atypical hemolytic uremic syndrome patients at a higher frequency than in a healthy population [12]. Three isoforms of C4BP with different subunit compositions have been identified in human plasma; the major isoform is comprised of seven α-chains and one β-chain (α7β1) while the other two isoforms are α7β0 and α6β1 [13]. The β-chain always carries anticoagulant, vitamin K dependent protein S [14]. C4BP is an acute phase protein, and its normal levels of around 220 μg/ml can be up-regulated around 4-fold [15].

3. C4BP is captured by many pathogens.

C4BP binds to a number of microorganisms and this number is constantly increasing (Table 1). In some cases the binding was correlated with resistance of bacteria to complement-mediated killing. Inhibition of complement by C4BP leads to decreased opsonisation of the bacteria with C3b, which in turn results in a decrease in phagocytosis that is the major weapon against the pathogens (Fig. 2). The number of pathogens (bacteria, yeast, parasites, viruses) that are able to bind or produce complement inhibitors is increasing and it can be speculated that all pathogens that must at some stage survive contact with blood are able to protect themselves by this mechanism. Another aspect of the observation that many pathogens bind to complement inhibitors is that the use

of complement inhibitors to prevent tissue rejection during xenotransplantation may predispose such individuals to infections.

Streptococcus pyogenes (group A *Streptococcus*) is one of the most common causes of bacterial infections in humans and can bring about a wide array of illnesses such as pharyngitis, impetigo, necrotising fasciitis, septicemia and toxic shock syndrome sometimes followed by rheumatic fever or glomerulonephritis. Important virulence factors, M proteins have been studied extensively due to their important ability to inhibit phagocytosis allowing bacteria to multiply in blood [16, 17]. A remarkable property of M proteins is their ability to bind a number of plasma proteins including C4BP [18] and FH [19]. Studies of several different M proteins showed that the high-affinity binding site for C4BP is localized to the hypervariable N-terminal region [20]. 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 [21]. M proteins interact with CCP1-CCP2 of α-chain (Fig. 3) [22, 23] and recently, structure of complexes between C4BP and M-proteins was described [24, 25]. The interaction with C4BP is restricted to primates [22, 26], 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 [27, 28]. 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 [28].

Furthermore, *Escherichia coli* K1 responsible for meningitis in neonates bind C4BP [29]. 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 [30]. It was subsequently shown that outer membrane protein A (OmpA) confers serum resistance both *in vivo* and *in vitro* [31], which appears to be related to the fact that CCP3 of C4BP α-chain interacts hydrophobically with the N-terminal part of OmpA [29]. 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. Furthermore, log phase OmpA+ *E. coli* K1 avoid serum bactericidal activity more effectively than postexponential phase bacteria as the former show increased binding of C4BP [32]. 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 [33].

Filamentous hemagglutinin from *Bordetella pertussis*, an etiologic factor of a whooping cough, is another surface protein known to interact with C4BP [34]. The binding is very similar to that between C4BP and C4b and may be an example of a molecular mimicry. The interaction is based on ionic interactions and requires a cluster of charged amino acids at the CCP1/CCP2 interface of the α-chain [35].

Candida albicans is the most common human pathogenic yeast causing cutaneous and mucocutaneous candidiasis [36]. In healthy individuals the cellular form of the yeast is often present as a commensal. However, *C. albicans* can also cause life threatening systemic infections especially in immunocompromised and granulocytopenic patients [37]. *C. albicans* activates all three pathways of the complement, but both yeast and hyphal forms of *C. albicans* capture complement

inhibitors FH and factor H-like protein 1 [38] as well as C4BP [39]. 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 [39]. Recently, binding of C4BP to a pathogenic mold (*Aspergillus*) was also reported [40].

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 [41]. C4BP binds ionically via CCP2 and CCP7 to ubiquitous surface proteins 1 and 2 (Usp1, Usp2) of *Moraxella* with Usp2 being the major binder [42]. Interestingly, Usp2 mediates serum resistance of the bacteria, which could be due, at least partially, to the binding of C4BP.

An interaction between C4BP and another important respiratory pathogen, non-typeable *Haemophilus influenzae* (NTHi), was also identified. Interestingly, the majority of the typeable *H. influenzae* (a-f) tested showed no binding [43]. Importantly, a low C4BP-binding isolate (NTHi 69) showed an increased deposition of C3b followed by reduced survival as compared with high-binding NTHi 506 when exposed to NHS. The binding is mediated by CCP2 and CCP7 of C4BP α-chains. Notably, C4BP bound to the surface of *H. influenzae* retained its cofactor activity as determined by analysis of C3b and C4b degradation.

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 [44] in a similar way to that of Lyme disease pathogen, *B. burgdorferi sensu stricto* [45, 46]. Furthermore, the relapsing fever spirochetes specifically bind C4BP [44] and both complement inhibitors retain their functional activities when bound to the surfaces of the spirochetes.

Two *Neisseria* species were shown to bind C4BP so far – *N. meningitidis* and *N. gonorrhoeae*, the latter being the main subject of this review. *N. meningitidis* (meningococcus) is an important cause of meningitis and sepsis. Host defense against neisseriae 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, lipooligosaccharide (LOS) sialylation, and/or expression of either porin (Por) A 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% [47]. 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.

4. Gonorrhea – the disease

N. gonorrhoeae is a gram-negative diplococcus and one of the two bacterial pathogens involved in the majority of cases of sexually transmitted genital infection and pelvic inflammatory disease (PID). *N. gonorrhoeae* can also cause disseminated gonococcal infection (DGI), which produces systemic manifestations. Gonorrhea is a significant health problem with over 60 million cases estimated to occur annually worldwide [48]. In addition to sequelae such as infertility and ectopic pregnancy, gonorrhea can enhance HIV co-transmission [49]. Antibiotic resistance in *N. gonorrhoeae* is currently a growing problem. The global spread of quinolone resistant *N. gonorrhoeae* is a serious predicament and has limited treatment options with oral antibiotics. Taken together, there is an urgent need to develop a vaccine against this pathogen. Understanding how gonococci resist killing by the complement system should prove invaluable in developing antibody-based vaccines against gonorrhea.

5. *N. gonorrhoeae* and complement evasion

Gonococcal strains that cause DGI usually are intrinsically resistant to the bactericidal action of nonimmune NHS [50] while those strains that cause PID are most commonly sensitive to killing by NHS *in vitro* [51]. Initially all gonococci that are recovered from the human genital tract are resistant to the bactericidal activity of NHS, but they may lose this property upon serial subculture [52]. The addition of CMP-sialic acid to growth media results in sialylation of LOS and reversion back to a resistant phenotype [53]. This is termed unstable serum resistance, and is usually mediated by binding of FH to sialylated organisms [54]. Because gonococci are heterogenously sialylated *in vivo* [6, 55], they may require mechanisms other than LOS sialylation in order to maintain the serum resistant state to enable them to survive in the human body. Binding of FH to porin is one such important serum resistance mechanism [56] providing protection from the alternative pathway of complement. Additionally, binding of C4BP allows *Neisseria* to down-regulate the two remaining complement pathways – the classical and the lectin routes.

6. Interaction of C4BP with *N. gonorrhoeae* and its functional consequences

6.1. C4BP binding correlates with serum resistance of *N. gonorrhoeae.*

In our initial study we screened 29 clinical and laboratory gonococcal isolates for C4BP binding from NHS using flow cytometry. Of these, 11 strains belonged to the Por1A serogroup, while the remaining 18 were Por1B strains. Por is a 34-35 kD protein comprising 8 transmembrane loops and functions as a selective anion channel and is essential for survival of the organism [57]. Por is the most abundant gonococcal outer membrane protein, and gonococci are classified into Por1A or Por1B serotypes and further into sero-subtypes or serovars based on the reactivity of a panel of monoclonal antibodies (mAbs) with Por [58]. We found that 10 of 11 Por1A strains were resistant to the bactericidal activity of 10% nonimmune NHS and that 8 eight of the 10 serum resistant Por1A strains bound C4BP, while the only serum-sensitive Por1A strain did not bind C4BP [59]. Eleven of the 18 Por1B strains were serum resistant, and of these 8 bound C4BP; 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 [60] we consequently detected strict correlation between binding of C4BP and serum resistance of *N. gonorrhoeae.*

Prior work has defined FH binding to the 5th loop of Por1A strains as a probable mechanism of stable serum resistance [56]. We then demonstrated that C4BP binding to Por1A strains served as

an additional mechanism that enabled these strains to evade complement. However, Por1B isolates form a significant proportion of gonococcal isolates worldwide [61, 62]. Por1B strains are generally nonbinders or weak binders of FH, and must therefore evade complement by other mechanisms. The ability of several Por1B strains to bind C4BP constitutes one such mechanism.

6.2. C4BP binds gonococcal porins

In order to characterize bacterial ligand to which C4BP was binding we replaced the Por1B molecule of a C4BP nonbinding strain with either the Por1A or the Por1B molecule of C4BP-binding strains. The isogenic mutant that expressed the Por molecule from a C4BP binder now bound C4BP, thus confirming that Por was the molecule that interacted with C4BP. Strains bearing hybrid Por1A/Por1B molecules showed that loop 1 of PorA1 was required, but not sufficient, for binding to C4BP. Therefore, the exact region in Por1A that binds to C4BP remains undefined. A region spanned by Por1B loops 5 and 7 was found to be necessary for C4BP binding [59]. An interesting observation was that all our Por1B strains that bound C4BP belonged to closely related serovars, and most bound to the serotyping mAb 3C8 [58] that is specific for a region encompassed by Por1B loops 5 and 6 [63]. This supported the notion that the central region of the Por1B molecule could be important in C4BP binding. Based on competition assays with C4b and heparin as well as by performing binding assays at different ionic strengths, we concluded that the C4BP-Por1B bond is ionic in nature [59], and that the binding site for Por1B in C4BP α-chain may reside at or very near binding sites for heparin and C4b, which previously has been mapped to a cluster of positively charged amino acids at the interface between CCP1 and 2 [64]. In contrast to the *N. meningitidis* PorA-C4BP ionic interaction that occurs optimally under hypotonic conditions, the *N. gonorrhoeae* Por1B-interaction proceeds unimpeded in normotonic buffers; decreased binding occurs in hypertonic buffers. The Por1A-C4BP interaction however, appears to be hydrophobic, and therefore is not influenced by ionic strength of buffers, heparin or C4b.

6.3. C4BP CCP1 contains the binding site for Por1A and Por1B

In order to determine the domain of C4BP that contained porin binding regions, we used recombinant C4BP molecules expressed in eukaryotic cells, which had individual α-chain CCPs (CCP1 through CCP7) deleted, and then studied their binding to strains FA19 (Por1A) and MS11 (Por1B). The C4BP mutant molecule lacking CCP1 did not bind to either FA19 or MS11 implying that CCP1 is required for binding to both gonococcal Por types [59]. Deletion of other domains individually had no significant impact on C4BP binding to gonococci. Further proof that CCP1 contained Por1A as well as Por1B binding sites was evidenced by showing that 5 mAbs directed against the *N*-terminal end of the α-chain of C4BP each could completely inhibit C4BP binding to strains FA19 and MS11. Accordingly, mAb 67, which is directed against C4BP α-chain CCP4, did not influence C4BP binding to either strain. Only human and chimpanzee C4BP bind to Por1B-bearing gonococci, while only human C4BP binds to Por1A strains (species specificity of C4BP binding is discussed below). We have utilized these species-specific differences in C4BP binding to gonococci to map the binding sites on CCP1 of C4BP. A comparison between human and chimpanzee or rhesus C4BP CCP1 revealed differences at 4 and 12 amino acid positions, respectively. These amino acids were targeted in the construction of 13 recombinant mutants of human C4BP. We found that amino acids T43, T45 and K24 individually, and A12, M14, R22 and L34 together, were important for binding to Por1A strains [60]. Altering D15 (found in man) to N (found in rhesus) introduced a glycosylation site that blocked binding to Por1A gonococci. C4BP

binding to Por1B strains required K24 and was partially shielded by additional glycosylation in the D15N mutant. Only those recombinant mutant C4BPs that bound to bacteria rescued them from killing by rhesus serum, thereby providing a functional correlate for the binding studies and highlighting C4BP function in gonococcal serum resistance.

6.4. Lipooligosaccharide (LOS) glycans modulate C4BP interactions with Por.

LOS heptose (Hep) glycan substitutions influence gonococcal serum resistance. We showed that the proximal glucose on HepI was required for C4BP binding to Por1B-bearing gonococcal strains MS11 and 1291 but not to FA19 (Por1A) [65]. The presence of only the proximal glucose on HepI (*l*ipooligosaccharide *g*lycosyl *t*ransferase E, or *lgt*E mutant) permitted maximal C4BP binding to Por1B strain MS11 but not to another Por1B strain called 1291. Replacing 1291 lgtE Por with MS11 Por increased C4BP binding to levels that paralleled MS11 lgtE, suggesting that the Por1B molecule dictated the effects of HepI glycans on C4BP binding. The remainder of the strain background did not affect C4BP binding; replacing the Por of strain F62, a C4BP nonbinder, with MS11 Por (F62 PorMS11) and truncating HepI (*lgt*E mutant) mirrored the findings seen in the MS11 background. C4BP binding correlated with resistance to killing by NHS in most instances. F62 PorMS11 and its lgtE mutant were sensitive to NHS despite binding C4BP, likely secondary to kinetically overwhelming classical pathway activation and possibly increased alternative pathway activation (the latter measured by factor Bb binding) seen with the F62 background. FA19 lgtF (HepI unsubstituted) resisted killing by only 10% NHS, but not 50% NHS, despite binding levels of C4BP similar to that seen with FA19 and FA19 lgtE (both resistant to 50% serum), suggesting a role for the proximal glucose in serum resistance independently of C4BP binding. These data identified another variable that modulates complement processing by *N. gonorrhoeae*, and highlighted the complex and intricate means utilized by this organism to evade the innate immune system.

6.5 C4BP binds to pili

Using a microtiter plate based assay and surface plasmon resonance technology (Biacore) we could demonstrate a direct, dose-dependent and saturable binding of C4BP to isolated type IV pili from *N. gonorrhoeae* [66]. The pili are elongated structures extending from the bacterial surface and in their absence the bacteria are not able to establish infection [67, 68]. Half-maximal binding of C4BP to immobilized pili occurred at 20 nM. We detected significant difference in C4BP binding between variants of a strain with or without pili; the tested strain (MS11) expressed a porin molecule that could bind C4BP, which contributed to a background level of C4BP binding. The binding between pili and C4BP was abolished in the presence of 0.25 M NaCl or C4b and was localized to CCP1-2 of C4BP α-chain. Type IV pili of pathogenic *N. gonorrhoeae* consist of a major pilus subunit protein, PilE, a minor pilus-associated protein PilC and possibly other as yet unidentified components [69, 70]. We found that the N-terminal part of PilC appeared to be responsible for binding of C4BP. Apart from binding C4BP, the pili use another complement inhibitor CD46 as receptor allowing them to enter epithelial cells [71]. CD46 competed with C4BP for binding to pili only at high concentrations, suggesting that different parts of pili are involved in these two interactions [66]. Accordingly, high concentrations of C4BP were required to inhibit binding of *N. gonorrhoeae* to Chang conjunctiva cells and no inhibition of binding was observed with cervical epithelial cells. However, presence of pili does not correlate with serum resistance and therefore the physiological relevance of the C4BP-pili interaction is not clear. C4BP bound to pili

could perhaps increase interaction of *Neisseria* with cellular surfaces as C4BP binds to several ligands such as heparan sulphate, CD91 [72], CD40 [73] and exposed phosphatidylserine [74] as has been shown for adenoviruses targeting liver [75]. However, this remains to be studied.

6.6. C4BP bound to gonococcal Por retains its function.

C4BP regulates classical complement pathway activation by facilitating inactivation of C4b by FI, thereby yielding C4d (remains attached to the bacterial surface) and C4c (released into solution). Therefore, C4BP cofactor activity will not alter the amount of C4d detected on the bacterial surface, but will result in a decrease in the amount of C4c bound to the organism, and result in a higher C4d/C4c ratio. We observed that C4BP-binding strains FA19 and MS11 showed a higher C4d/C4c ratio (≈4) than that observed with strain F62 (≈1.2) that does not bind C4BP [59]. Thus, C4BP bound directly to the bacterial surface exhibits cofactor activity. We demonstrated that fAb fragment of mAb 104 that blocks binding of C4BP to gonococci, when added to NHS, abrogates serum resistance of MS11 [59]. As a control we used fAb fragments derived from mAb 67 (binds to C4BP CCP4 and does not block interaction) in this assay and observed no effect on bacterial viability. Thus, even though the binding sites for porins and C4b overlap on C4BP it does not decrease activity of C4BP while bound to bacterial surface. This is due to the fact that C4BP is a polymeric molecule with seven identical α-chains each carrying the same binding sites. Therefore, C4BP can use overlapping or even identical binding sites to engage with many ligands at the same time, which is a rather unusual property for a plasma protein.

6.7. Binding of C4BP to *N. gonorrhoeae* is species specific

We found that 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* [76]. In contrast, we found that *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 [77] 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 [78]. 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 neisserial vaccine candidates.

7. Conclusions

Complement forms an important arm of innate immune defenses against infections, both in the bloodstream and on mucosal surfaces. Several pathogens, including *N. gonorrhoeae*, bind C4BP and subvert complement attack. The ability of *N. gonorrhoeae* to evade complement appears to be critical to its survival in the host. *N. gonorrhoeae* is a pathogen that is uniquely adapted to survival in humans and at least one factor that contributes to human-limited disease could be because *N. gonorrhoeae* binds only human (and in the case of certain Por1B strains, chimpanzee) C4BP. These findings have implications for developing better animal models to study gonococcal infections – for

example, a mouse that transgenically expresses human C4BP may be more susceptible to gonococcal infection. Over the past several decades, *N. gonorrhoeae* has demonstrated an extraordinary ability to develop resistance to antibiotics. There is a need to develop newer therapeutic agents and a vaccine against this disease. Blocking C4BP-gonococcal interactions either by vaccine-elicited antibodies or by therapeutic molecules may provide avenues to better combat this disease.

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Table 1 Pathogens, which were identified to bind human complement inhibitor C4BP.

Pathogen	Disease	Surface ligand	Binding site (C4BP)	Type of binding	Reference
Neisseria gonorrhoeae	gonorrhea, disseminated gonococcal infection	porin 1A (loop 1) porin 1B (loops 5, 6) type IV pili (pilC)	CCP1 CCP1 CCP1-2	hydrophobic ionic ionic	[59, 66]
Neisseria meningitides	meningitis	porin A	CCP2-3	ionic	[47]
Bordetella pertussis	whooping-cough	hemagglutinin and ?	CCP1-2	ionic	[35]
Streptococcus pyogenes	strep throat, necrotizing fasciitis, rheumatic fever	M proteins (hypervariable region)	CCP1-2	hydrophobic	[23, 28]
Escherichia coli K1	neonatal meningitis	OmpA : Outer membrane protein A (N-terminus)	mainly CCP3, CCP8	hydrophobic	[29]
Moraxella catarrhalis	otitis media, sinusitis	Usp1, 2: Ubiquitous surface protein 1 and 2	CCP2, CCP7	hydrophobic	[42]
Borrelia recurrentis and *duttonii*	relapsing fever	?	?	?	[44]
Candida albicans	candidiasis in immuno-compromised	?	CCP1-2, CCP6	ionic	[39]
Aspergillus spp	systemic infections in immunocompromised	?	?	?	[40]
Yersinia pestis	plaque	?	?	?	[76]

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Figure legends

Figure 1. Scheme of the complement system and its inhibitors. Three pathways by which the human complement system can be activated and their physiological effects: clearance of apoptotic cells, opsonization of pathogens and immune complexes for phagocytosis, release of anaphylatoxins and lysis. Furthermore, sites of action of soluble and membrane-bound complement inhibitors are indicated. The majority of inhibitors act on C3-convertases while C1-inhibitor controls activation of C1 complex and CD59 inhibits MAC formation.

Figure 2. Pathogens capturing C4BP are protected from complement mediated lysis and phagocytosis. C4BP bound to the surface of a pathogen inhibits surface-bound classical C3-convertase and serves as FI cofactor in cleavage of C3b in solution as well as C4b both in solution and surface-bound, which leads to decrease in opsonisation and less efficient phagocytosis. Furthermore, assembly of MAC and lysis are also inhibited. Importantly, C4BP is a multimeric protein that is able to interact with several ligands simultaneously even if they occupy overlapping binding sites.

Figure 3. Schematic representation of C4BP with indicated binding sites for various 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. C4BP interacts with a number of bacterial ligands but also with endogenous proteins such as C4b, C3b, heparin, DNA, CD91, serum amyloid P component (SAP) and C-reactive protein (CRP). The β-chain engages only one known ligand, namely protein S.

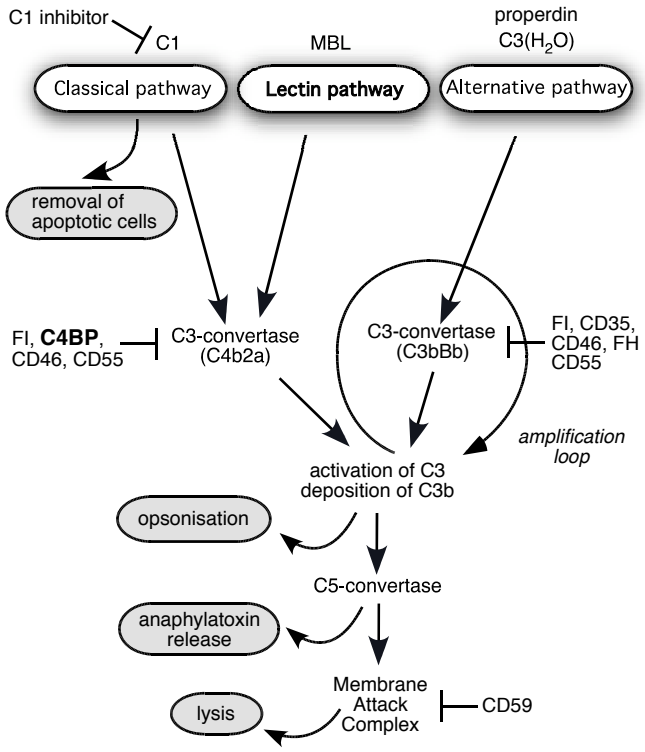

Fig. 1, Vaccine, Blom

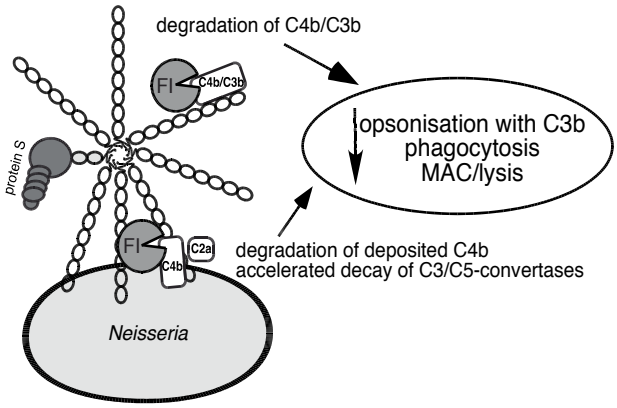

Fig. 2, Vaccine, Blom

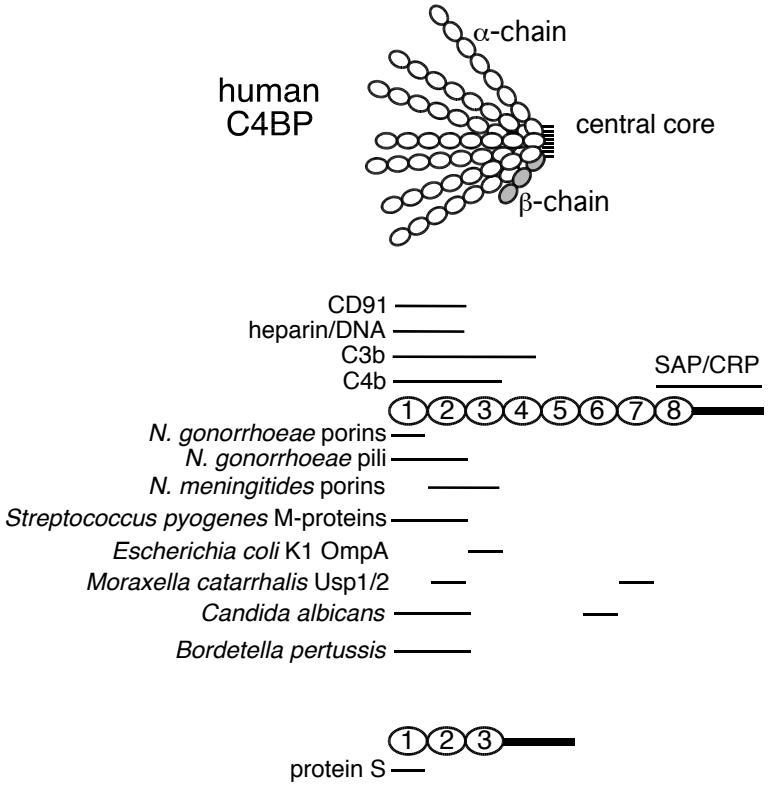

Fig. 3, Vaccine, Blom