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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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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 porin proteins 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 the 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

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.

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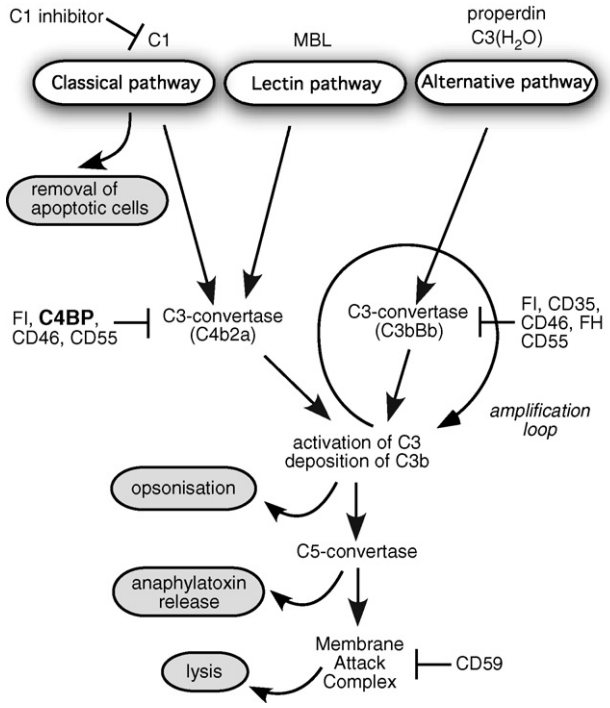

Fig. 1. Scheme of the complement system and its inhibitors. Three pathways by which the human complement system can be activated and their physiological effects: clearance of apoptotic cells, opsonization of pathogens and immune complexes for phagocytosis, release of anaphylatoxins and lysis. Furthermore, 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.

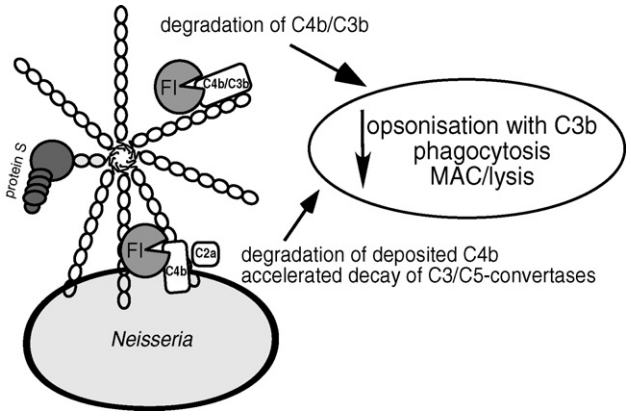

Fig. 2. Pathogens capturing C4BP are protected from complement-mediated lysis and phagocytosis. C4BP bound to the surface of a pathogen inhibits surface-bound classical C3-convertase and serves as FI cofactor in cleavage of C3b in solution as well as C4b both in solution and surface-bound, which leads 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.

of C4b and it also accelerates the decay of the classical pathway C3 convertase (C4bC2a) [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-chain 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].

Complete 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 fourfold [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

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	Porin 1A (?loop 1)	CCP1	Hydrophobic	[59,66]
	Gonococcal infection	Porin 1B (loops 5, 6)	CCP1	Ionic	
		Type IV pili (pilC)	CCP1-2	Ionic	
Neisseria meningitidis	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 immunocompromised	?	CCP1-2, CCP6	Ionic	[39]
Aspergillus spp.	Systemic infections in immunocompromised	?	?	?	[40]
Yersinia pestis	Plague	?	?	?	[76]