The Hijackers Guide to Escaping Complement: lessons learned from pathogens

David Ermert [a,b], Sanjay Ram [c] and Maisem Laabei [d]

[a] Department of Translational Medicine, Division of Medical Protein Chemistry, Lund University, Malmö, Sweden

[b] Department of Preclinical Research, BioInvent International AB, Lund, Sweden

[c] Department of Medicine, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

[d] Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom

Corresponding author: Maisem Laabei; ml418@bath.ac.uk

Abstract

Pathogens that invade the human host are confronted by a multitude of defence mechanisms aimed at preventing colonization, dissemination and proliferation. The most frequent outcome of this interaction is microbial elimination, in which the complement system plays a major role. Complement, an essential feature of the innate immune machinery, rapidly identifies and marks pathogens for efficient removal. Consequently, this creates a selective pressure for microbes to evolve strategies to combat complement, permitting host colonization and access to resources. All successful pathogens have developed mechanisms to resist complement activity which are intimately aligned with their capacity to cause disease. In this review, we describe the successful methods various pathogens use to evade complement activation, shut down inflammatory signalling through complement, circumvent opsonisation and override terminal pathway lysis. This review summarizes how pathogens undermine innate immunity: *'The Hijackers Guide to Complement'*.

1. Introduction

The complement system represents a sophisticated, evolutionarily conserved pathway composed of over 50 fluid-phase and membrane-bound components. Complement is an essential part of innate immunity and works in concert with phagocytes to survey, label and destroy microbial intruders (Berends et al., 2014; Merle et al., 2015). Additionally, complement is central in coordinating inflammation, immune surveillance and recycling and eliminating cellular debris, thereby maintaining homeostasis (Markiewski and Lambris, 2007; Ricklin et al., 2010). Finally, complement interacts with the adaptive immune system; interaction of C3 fragments with B-cells lowers their activation threshold, and C3 and C5 fragments modulate intracellular metabolic reprogramming of T cells to influence downstream immune signalling pathways (Elvington et al., 2016; Killick et al., 2018).

Complement can be activated by three different routes, the classical (CP), lectin (LP) and alternative (AP) pathways (Figure 1, left side and middle top), each with distinct initiating mechanisms which enable recognition of diverse structures (Berends et al., 2014; Merle et al., 2015). The CP is governed by the recognition of pre-bound immunoglobulins (Ig), IgG and IgM or specific pathogen associated molecular patterns (PAMPs) by C1q (Merle et al., 2015; Noris and Remuzzi, 2013). Binding of C1q (C1q, C1r and C1s exist as a complex called the C1 complex) to its ligands results in a conformational change which initiates auto-activation of accompanying serine protease, C1r, which cleaves and activates neighbouring serine protease C1s. Activated C1s targets C4 generating C4a and C4b. The function of C4a is a poorly understood, however C4b reacts with amino and hydroxyl groups on surfaces via the exposed thioester domain (Law and Dodds, 1997). C2 now interacts with C4b, and is cleaved by C1s to generate C2a and C2b. The larger C2a fragment interacts with C4b to form the CP C3 convertase, C4b2a (Merle et al., 2015; Noris and Remuzzi, 2013).

LP activation is initiated when mannose-binding lectin (MBL), ficolins (ficolin-1, -2 or -3) or collectin-11 interacts with select carbohydrate moieties displayed on microbes. Similar to C1q, these recognition molecules are complexed with homologous proteases, termed MBL-associated serine proteases (MASPs). MASPs become activated following interaction of LP initiators with microbial surfaces. MASP-2 specifically cleaves C4, while both MASP-1 and MASP-2 are responsible for C2 cleavage, generating a C3 convertase identical to the CP (Garred et al., 2016; Merle et al., 2015).

Activation of the AP results from the spontaneous hydrolysis of a labile thioester bond present in the C3 molecule, generating a biologically active conformation termed C3(H_2O) (Harboe and Mollnes, 2008; Merle et al., 2015; Noris and Remuzzi, 2013). Hydrolysis of C3 to C3(H_2O) can be accelerated via interactions between C3 and biotic and artificial interfaces (Nilsson and Nilsson Ekdahl, 2012). The exposed thioester domain of C3(H_2O) permits binding of Factor B (FB), resulting in the formation of an efficient substrate for the serine protease Factor D (FD) which cleaves FB into Ba and Bb, generating the fluid-phase C3 convertase, C3(H_2O)Bb (Bexborn et al., 2008). C3(H_2O)Bb can cleave C3 into C3a and C3b, allowing C3b to bind covalently to surfaces containing exposed hydroxyl groups including microbial surfaces (Sahu et al., 1994).

All complement pathways converge at the level of C3 convertase formation, efficiently processing C3 into C3a and C3b (Figure 1, centre). Deposition of C3b triggers the AP positive feedback loop, thus enabling the AP to amplify C3b deposited by the CP and LP (Lachmann, 2009). Analogous to C3(H_2O), deposited C3b interacts with FB, is processed by FD, forming AP C3 convertase, C3bBb. Importantly, any deposited C3b may interact with FB and FD resulting in amplification of surface bound AP C3 convertase and C3b deposition. While C3a is an important inflammatory mediator, iC3b (generated by C3b cleavage) is the central opsonin. Opsonisation of pathogens with C3 cleavage fragments is an efficient method to label microbes for phagocyte-mediated uptake and subsequent destruction. The only known positive regulator of complement, properdin, prolongs the life of AP C3 convertases by stabilizing the interaction between C3b and FB. Properdin may also provide a platform for C3bBb surface assembly (Hourcade, 2006).

Continued C3b deposition on the microbial surfaces promotes a change in convertase function from cleaving C3 to preferentially cleaving C5, via the assembly of the CP/LP C4b2aC3b and AP C3bBbC3b C5 convertases (Merle et al., 2015; Noris and Remuzzi, 2013). Cleavage of C5 generates C5a, a potent anaphylatoxin and C5b, an integral membrane attack complex (MAC) component. C5b interacts with multiple complement proteins in a step wise manner. Formation of C5b-7 permits interaction with cell membranes, while incorporation of C8 promotes insertion into the lipid bilayer. Lastly, the inclusion of multiple C9 molecules results in the formation of a tubular MAC pore which can lyse susceptible cells such as gram-negative bacteria (Bayly-Jones et al., 2017; Ricklin et al., 2010). Recent work has demonstrated

that bacterial killing by MAC requires local, C5 convertase-mediated assembly of C5b-6 to permit efficient insertion of MAC into bacterial membranes (Heesterbeek et al., 2019).

Several host soluble or cell-surface attached complement inhibitors limit the destructive effects of complement on 'self' surfaces (Merle et al., 2015; Noris and Remuzzi, 2013; Zipfel and Skerka, 2009). Membrane-bound regulators include decay-accelerating factor (DAF/CD55), membrane cofactor protein (MCP/CD46), complement C3b/C4b receptor 1 (CR1/CD35) and CD59. With the exception of CD59, all these regulators contain complement control protein (CCP) domains which interact with their specific ligands. The primary targets of most complement regulators are C3b, C4b or C3 convertases; CD59 limits C9 polymerization (Noris and Remuzzi, 2013; Zipfel and Skerka, 2009).

Soluble negative regulators in plasma also dampen complement activity. C4b-binding protein (C4BP) limits the function of C4b and is a potent inhibitor of both the CP and LP. C4BP acts as a cofactor for FI mediated cleavage of surface-bound and soluble C4b and also hastens the natural decay of C3 convertases (D. Ermert and Blom, 2016). The master regulators of the AP are Factor H (FH) and Factor H-like protein 1 (FHL-1), an alternatively spliced transcript of FH (Ferreira et al., 2010). These regulators serve as cofactors for C3b cleavage by FI, compete with FB for interaction with C3b and promote C3bBb dissociation. Another soluble regulator, C1 inhibitor (C1-INH), functions as a serine protease inhibitor targeting and inactivating C1r, C1s, MASP-1 and MASP-2 and therefore disrupting both CP and LP activity (Davis et al., 2010).

Two plasma proteins, clusterin and vitronectin, reduce 'innocent bystander' lysis of host cells by MAC (Preissner, 1991; Tschopp et al., 1993). Additionally, the membrane bound regulator CD59, interacts with both C5b-8 and C5b-9 to prevent MAC penetration into the lipid bilayer (Huang et al., 2006). Importantly, CD59 does not bind to circulating C8 or C9 but specifically to the MAC/perforin (MACPF) domain of each protein upon complex formation (Wickham et al., 2011).

While several new functions have been attributed to complement over the past twenty years, the 'basic' anti-pathogen role of complement remains critical for human health, highlighted by the heightened risk of certain infections in patients with complement deficiencies (J. E. Figueroa and Densen, 1991; Ram et al., 2010). Infectious diseases represent one of the most serious threats to global human health, a problem amplified by the increasing rate of antibiotic resistance and a concomitant stagnation in antibiotic and vaccine development. Accordingly, there is an urgent need to develop therapeutic interventions to

combat infection. This requires a greater understanding of the complex interplay between host and pathogen. The purpose of this review is to provide an update of essential complement evasion strategies used by some of the important human pathogens, which could provide new avenues to target pathogens.

1.1 Importance of complement against infection – observations in complement deficient individuals

Complement deficiencies may be inherited or acquired. Acquired complement defects may occur following acute infections or may accompany chronic disease states such as cirrhosis or protein-losing nephropathies. Acquired complement deficiencies also result from therapeutic blockade of the complement cascade as with the drug eculizumab (C5 inhibitor) and will be encountered more frequently in the near future as an increasing number of therapeutic complement inhibitors enter clinical trials (Ricklin and Lambris, 2016; Ricklin et al., 2018)). Congenital deficiencies in the complement system are rare and are diagnosed when individuals present with certain autoimmune diseases or recurrent bacterial infections (Ram et al., 2010; Ricklin and Lambris, 2016).

Individuals with defects of components of the CP are predisposed to autoimmune disorders (systemic lupus), which presents at an early age (Barilla-LaBarca and Atkinson, 2003; Lintner et al., 2016; Ricklin and Lambris, 2016). These individuals have a relatively low frequency of infection (20%), attributable to a functional AP. Infections in this population are often caused by encapsulated bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, which involve the sinuses, respiratory tract, blood or meninges (J. E. Figueroa and Densen, 1991; Ram et al., 2010). Individuals with deficiencies of FD and properdin are predisposed to meningococcal disease (Biesma et al., 2001; Fijen et al., 1999b; Mathew and Overturf, 2006; Sprong et al., 2006). A single family with FB deficiency has been described; the index case suffered recurrent episodes of invasive pneumococcal infection and an episode of meningococcal disease (Slade et al., 2013). Consistent with its central role in the complement cascade, persons with C3 deficiency contract pneumococcal and meningococcal infections at an early age (J. E. Figueroa and Densen, 1991). Persons with deficiencies in terminal complement proteins exclusively suffer from 7,000-10,000-fold higher rates of recurrent invasive meningococcal disease than their complement-sufficient counterparts (J. Figueroa et al., 1993; J. E. Figueroa and Densen, 1991; Fijen et al., 1999a; Lewis and Ram, 2014). A

relatively mild course and lower mortality characterize meningococcal infections in terminal complement deficient persons (J. E. Figueroa and Densen, 1991). MAC insertion into gram-negative membranes results in lipopolysaccharide (LPS; endotoxin) release (O'Hara et al., 2001; Tesh et al., 1986); the amount of complement activation correlates with endotoxin release and disease severity (Brandtzaeg et al., 1989). Conversely, lack of the ability to effectively form the MAC pore limits the amount of endotoxin released (Lehner et al., 1992) and consequently such individuals enjoy a lower mortality per meningococcal disease episode. Eculizumab use is also associated with a ~2000-fold higher risk of invasive meningococcal infection; most reported cases were caused by unencapsulated (non-groupable) isolates (McNamara et al., 2017). Eculizumab treatment has also been associated with several cases of disseminated gonococcal infection (Crew et al., 2018) and infections caused by otherwise non-pathogenic commensal Neisseria species (Crew et al., 2019). Collectively, these epidemiologic observations highlight the key role for complement in combating infections and in particular, invasive Neisserial infections.

2. Manipulation of AP

2.1 Targeting properdin

Properdin, the only positive regulator of complement, functions primarily to stabilize the AP C3 convertase, C3bBb. In the absence of properdin, C3bBb dissociates quickly ($T_{1/2}$ ≈90 sec), which is increased 5- to 10-fold when properdin associates with C3bBb (reviewed in (Kemper and Hourcade, 2008)). A stable and active C3b convertase increases opsonisation of pathogens. As a result, certain bacterial species have evolved strategies to interrupt this stabilising function. LPS are integral components of the gram-negative cell membrane, are crucial for membrane stability and serve as a physical barrier from environmental factors. LPS is a negatively charged molecule composed of hydrophobic lipid A anchored in the membrane, which is linked to a hydrophilic inner oligosaccharide core. Several gram-negative pathogens possess glycan extensions organized in repeating units beyond the inner oligosaccharide core called the O-antigen (Steimle et al., 2016). In certain bacterial species, LPS has been suggested to prevent properdin binding to the bacterial surface. Isogenic LPS mutants of either the O-antigen or core oligosaccharide in *Escherichia* coli K12 display enhanced levels of both properdin and C3b deposition compared to wild type, however the exact mechanism of how LPS thwarts properdin binding is not fully understood (Spitzer et al., 2007).

Another strategy employed by bacteria is the direct degradation of properdin. *Streptococcus pyogenes* expresses a secreted virulence factor called *Streptococcus pyogenes* exotoxin B (SpeB). This cysteine protease SpeB directly cleaves and inactivates properdin (Tsao et al., 2006), and several other complement proteins (Laabei and Ermert, 2019) and is discussed below.

2.2 Blocking AP convertase

Staphylococcus aureus, a master of complement evasion, uses a wide variety of different complement evasion strategies including potent secreted anti-convertase molecules (reviewed in (Lambris et al., 2008)). Staphylococcal complement inhibitors (SCIN, SCIN-B/C), arrest both CP/LP and AP C3 convertases in a non-functional conformation (Jongerius et al., 2007; Rooijakkers et al., 2005). AP C3 convertase inhibition has been better characterized, where SCIN binds to both C3b/iC3b and Bb, forming a bridge between C3b and Bb, locking Bb into a non-active state (Rooijakkers et al., 2009). Extracellular fibrinogen binding protein (Efb) and homologue of the C-terminus of Efb, Extracellular complement binding protein (Ecb), specifically stabilise the C3bB proconvertase on the bacterial surface via interaction with C3b. Efb/Ecb binding to C3b enhances FB-C3b contact which prevents FB cleavage by FD. In addition, Ecb/Efb also effectively block C5 convertase activity through interaction with C3d (Hammel et al., 2007a; Hammel et al., 2007b; Jongerius et al., 2010; Lee et al., 2004).

2.3 Acquisition of FH

FH binds to deposited C3b and select glycosaminoglycans (GAG; e.g., heparin sulfate, heparin dermatan- or chondroitin sulfate A) simultaneously on host cells through domains 19 and 20, respectively (Figure 2). This enables domains 1-4, which contains the complement inhibitory region of FH, available to bind to C3b (Gordon et al., 1995) and exert decay accelerating activity and FI cofactor activity. “Self/non-self discrimination” is crucial for sparing the host from unwanted complement activation and is mediated mainly through domains 19-20 (Pangburn, 2002). Another GAG binding region exists within a region spanned by domain 6 and 7; impaired binding of this region as seen with the 402H variant of FH domain 7 to malondialdehyde, a lipid peroxidation product, within the eye may promote the formation of drusen that is characteristic of the dry form of age-related macular degeneration (Weismann et al., 2011).

Interestingly, many microbes that have co-evolved with their hosts have developed the ability to bind to FH in a similar manner as their hosts. Therefore, it is not surprising that most microbes that bind FH do so through domains 6-7 and/or 19-20 (Figure 2; Suppl Table 1). Microbial proteins may form a tripartite interaction with FH domains 19-20 and C3b, similar to that between host glycosaminoglycans, C3b and the C-terminus of FH (Meri et al., 2013). *Neisseria gonorrhoeae* sialylated lipooligosaccharide (LOS) and outer membrane porin protein together mediate a stable interaction with the C-terminus of FH (Madico et al., 2007). Species selective binding of FH (as well as C4BP) might be one of the key reasons for a host specificity. Bacteria such as nontypeable *H. influenzae* (NTHi), *N. meningitidis*, *N. gonorrhoeae* or *S. pyogenes* cause natural infection only in humans and also preferentially bind human Factor H (D. Ermert et al., 2015; Granoff et al., 2009; Langereis et al., 2014; Ngampasutadol et al., 2008; Schneider et al., 2009). By contrast, *Borrelia burgdorferi* strains that infect diverse species are capable of recruiting FH from all their hosts (Hart et al., 2018). In addition to direct complement inhibition, FH can bind to the lipid A part of LPS of *E. coli* TG1 (Tan et al., 2011). Bacteria-bound FH can compete with C1q for binding thus interfering not only with the AP, but also CP.

Members of the FH family, such as Factor H like protein (FHL) and - related proteins (FHR) also contribute to complement activation and regulation on microbial surfaces. FHL-1 contains the CCP domains 1-4 and can inhibit complement deposition. In contrast, the FHR proteins all lack the first 4 domains of FH and therefore lack FI cofactor activity. Thus, binding of FHR proteins to microbes – as an example, the FHR3 - *N. meningitidis* interaction (Caesar et al., 2014) - interferes with binding of FH/FHL-1 and serves to activate complement (reviewed in (Jozsi, 2017)). It is conceivable that FHR-1, which contains three C-terminal domains that are almost identical to FH domains 18-20 may also compete with binding of FH domains 19 and 20 to microbes. We speculate that the FHR family of proteins may have evolved to counteract the ability to microbes to steal FH from the host and evade complement.

2.4 Binding C3 and recruiting FI

The Herpes simplex virus type 1 (HSV-1) glycoprotein (gC1) is essential in resisting complement attack (Harris et al., 1990; J. Lubinski et al., 1999; J. M. Lubinski et al., 1998). gC1 interacts directly with C3 and C3 activation products, C3b, iC3b and C3c (Harris et al., 1990; Kostavasili et al., 1997). gC1 binds a distinct epitope on C3b blocking its interaction with properdin and

thus accelerating the decay of the AP convertase (Kostavasili et al., 1997; J. M. Lubinski et al., 1998).

Vaccinia, variola and monkey pox viruses all produce complement inhibitory proteins that possess CCP modules called vaccinia complement control protein (VCP), smallpox inhibitor of complement enzymes (SPICE) and monkeypox inhibitor of complement enzymes (MOPICE), respectively (Dunlop et al., 2003; Kotwal, 2000; Liszewski et al., 2006; Mullick et al., 2003). While all three proteins possess variable levels of FI cofactor activity for C3b and C4b, only VCP and SPICE, but not MOPICE possess decay-accelerating activity (Liszewski et al., 2006). A virulent strain of the Nipah virus possesses FI-like activity, which can cleave C3b in the presence of the cofactors, FH or CR1 (Johnson et al., 2015). A less virulent Nipah virus strain lacked this ability, which points to a role for complement inactivation in pathogenesis of this virus.

The human specific respiratory tract pathogen, *Moraxella catarrhalis*, has evolved multiple mechanisms to resist-complement mediated lysis, the majority of which rely on the expression of two membrane autotransporters, ubiquitous surface protein A (UspA1) and UspA2 (de Vries et al., 2009). A unique evasive strategy displayed by *M. catarrhalis* involves direct interaction between UspA2 and non-activated C3, resulting in neutralisation of C3 and inhibition of all complement pathways (Nordstrom et al., 2005).

S. aureus actively recruit FI to the bacterial surface, limiting C3 convertase formation and significantly diminishes phagocytosis (Hair et al., 2008). This mechanism is mediated through the expression of the multifunctional cell wall protein, clumping factor A (ClfA). ClfA is composed of a N-terminal ligand binding A region, followed by a serine-aspartate repeat domain and a C-terminal region that permits covalent anchorage to the peptidoglycan (Foster et al., 2014). FI interacts with the A domain. Furthermore it was noted that a recombinant fragment of ClfA that consisted mostly of the A domain exhibited cofactor activity for FI and enhanced cleavage of C3b to iC3b in the absence of known cofactors (Hair et al., 2008). Therefore, these data suggest that ClfA both recruits and localizes FI to the staphylococcal surface while simultaneously augmenting FI mediated cleavage of deposited C3b.

3. Evasive strategies directed at CP & LP

3.1 Disrupting Ab binding

Binding of the C1q component of the C1 complex to surface-bound IgM or IgG initiates CP activation. Efficient IgG-C1q complement activation relies on optimal antigen epitope distribution, which permits the formation of ordered IgG Fc hexamers, thereby providing a platform for high avidity Fc-gC1q interaction (Diebolder et al., 2014).

Because Ab-complement interactions on microbial surfaces are critical for their clearance, many human pathogens have evolved mechanisms to disrupt this interaction. One of the first bacterial immune evasion mechanisms described was the immunoglobulin binding protein, protein A, of *S. aureus* (Sjodahl, 1977). Protein A is a cell-wall anchored protein composed of five N-terminal triple-helical bundle domains which interact with several ligands including IgG (Foster et al., 2014; Moks et al., 1986). Specifically, protein A captures the Fcγ domain of human and multiple mammalian IgGs. Crucially, protein A is highly expressed on the staphylococcal surface and results in coating of the bacterium by IgG in an 'upside down' orientation (DeDent et al., 2007), which prevents the Fc region of IgG from engaging C1q or Fc receptors on professional phagocytes thereby impeding both, CP activation and phagocytosis.

Several bacteria and fungi express surface polysaccharide capsules. Capsules confer many benefits to the microbe including preventing desiccation and resisting innate and adaptive immune responses. Polysaccharide capsules are hydrated, highly variable homo- or heteropolymeric structures composed of repeating monosaccharides linked by glycosidic bonds, which can extend for up to 400 nm from the bacterial surface (Roberts, 1996). This variability gives rise to many distinct capsule serotypes – as an example, there are over 90 different capsular types (serotypes) of *S. pneumoniae* – which poses a moving target for the immune system. In a classic case of molecular mimicry, capsules composed of α2-8-linked homopolymers of N-acetylneuraminic acid elaborated by *Neisseria meningitidis* serogroup B and *E. coli* K1 are identical to human neural cell adhesion molecule (NCAM) and therefore poorly immunogenic (Roberts et al., 1989). Additionally, *N. meningitidis* capsule prevents engagement of C1q by antibodies directed against surface protein, which results in decreased C4b deposition (S. Agarwal et al., 2014). Capsule also impedes the AP-mediated C3b deposition by masking microbial targets for C3b (Roberts, 1996). Capsule may also prevent any C3b deposited on the membrane from binding to complement receptors on phagocytes. Alternatively, pathogens can modify their capsular polysaccharide content to evade complement. As an example, certain *Klebsiella pneumoniae* serotypes that lack mannobiose

and rhamnobiose and avoid recognition by the LP tend to be more virulent than their counterparts that express these two sugars (Sahly et al., 2009).

Capsule works in concert with other outer membrane molecules such as LPS to resist innate immunity. Because LPS is surface exposed it is readily recognized by antibodies. LPS can undergo structural changes; the presence of O-antigenic repeats results in the 'smooth LPS' phenotype while loss of O-antigen expression results in a 'rough LPS' phenotype (Steimle et al., 2016). In general, rough LPS strains are more susceptible to the bactericidal activity of complement than smooth LPS strains. In *K. pneumoniae*, elongated O-antigen limits C1q binding and subsequent C3b deposition; any deposited C3b is too far from the membrane to permit bactericidal MAC insertion into the lipid bilayer (Merino et al., 1992). Certain bacterial species lack the O-antigen and express lipooligosaccharide (LOS), which can be modified to evade complement. *N. gonorrhoeae* LOS contains a lacto-N-neotetraose (LNnT) moiety (also a host mimic (Mandrell and Apicella, 1993) which can be sialylated by an enzyme called LOS sialyltransferase (Gilbert et al., 1996). Sialyation of gonococci enables bacterial survival in serum (Smith et al., 1995). LOS sialylation interferes with all three pathways of complement. First, bacteria with sialylated LOS bind less IgG present in normal human serum (Gulati et al., 2015) or specific antibodies, such as anti-porin antibodies (Elkins et al., 1992). Second, MBL interaction with gonococci is decreased following sialyation (Devyatyarova-Johnson et al., 2000; Gulati et al., 2002). Finally, LOS sialyation represses AP activation (Ram et al., 2018; Ram et al., 1998); enhanced FH binding is restricted to sialic acid α2-3-linked to LNnT LOS (Ram et al., 1998).

3.2 Inhibition of C1 / C4

Targeting the C1 complex is an efficient method of disrupting CP activation. Recently a novel mechanism of CP evasion was shown for the Lyme disease spirochete, *Borrelia burgdorferi* (Garcia et al., 2016). *B. burgdorferi* utilises a surface expressed lipoprotein, BBK32, to capture C1 with high affinity. Specifically, BBK32 binds to C1r non-covalently in a calcium-dependent manner and prevents autoactivation and cleavage of C1s, thus maintaining C1 in its inactive proenzyme state. *S. aureus* also targets the interaction of C1q with the initiating serine proteases to prevent CP induction. To achieve this, *S. aureus* expresses a surface protein called collagen binding protein (Cna) (Kang et al., 2013), which interacts specifically with the collagenous domain of C1q and prevents its interaction with C1r. Moreover, Cna actively

displaces $C1r_2C1s_2$ from the C1 complex and also prevents C1 from interacting with IgM coated surfaces.

CP/LP evasion may also occur through the recruitment of C1-INH. C1-INH is a multi-functional acute-phase protein belonging to the superfamily of serine protease inhibitors (Serpins). This molecule contains a C-terminal protease recognition region or 'reactive loop' which mimics target proteases cleavage sites. Cleavage at the specific substrate site by target proteases triggers a conformational rearrangement in which C1-INH and the protease becomes irreversibly locked through covalent bonds blocking the protease active site ('suicide inhibition') (Davis et al., 2010). Complement specific targets of C1-INH include C1r, C1s, MASP-1 and MASP-2. *Bordetella pertussis*, the causative agent of whooping cough, employs a surface expressed autotransporter, Virulence associated gene 8 (Vag8), to bind C1-INH via its serpin domain, which enhances complement resistance (Marr et al., 2011). A secreted form of the passenger domain of Vag8 (the same domain that binds C1-INH) also prevents serum killing of pertussis (Hovingh et al., 2017). Mechanistically, recombinant passenger domain Vag8 or full-length secreted Vag8 binds C1-INH and prevents it from interacting with C1r, C1s and MASP-2 in solution. Loss of C1-INH function results in cleavage and consumption of C4 and C2 in solution (i.e., away from the bacterial surface), which represses normal CP/LP activity on the bacterial surface (Hovingh et al., 2017).

NS1 is a secreted glycoprotein expressed by several members of the Flaviviridae family of RNA viruses, including the dengue, West Nile and yellow fever viruses. In a novel strategy, soluble NS1 derived from these pathogens target the CP and LP by directly binding C4 and C1s, which results in enhanced cleavage of C4 to C4b in solution and therefore depletes the supply of C4 and prevents complement activation on the viral surface (Avirutnan et al., 2010).

3.3 Blockade of CP/LP convertase

Targeting CP/LP C3 convertase formation efficiently limits complement activity. Extracellular adherence protein (Eap) is one of a number of soluble *S. aureus* complement C3 convertase inhibitors (Thammavongsa et al., 2015). Eap is a multi-functional 60-72 kDa protein; different isoforms, consisting of four to six 110 amino acid repeats, exist (Hussain et al., 2001). Eap exhibits potent CP/LP C3 convertase inhibition activity, leading to reduced C3b deposition on the bacterial surface, thus inhibiting opsonophagocytic killing by neutrophils (Woehl et al., 2014). Eap domains 3 and 4 (Eap3-4) bind C4b with nanomolar affinity, which effectively

prevents subsequent C4b interaction with either full length C2 or C2b. Structural analysis revealed that Eap34 targets the α' and γ chains of C4b and highlighted seven key lysine residues required for C4b binding and complement inhibition (Woehl et al., 2017). It is worth noting that although Eap interacts with C4b at a site similar to C4BP, it neither interferes with the inhibitory activity of C4BP nor displays intrinsic cofactor activity for FI-mediated C4b degradation.

The *Schistosoma* parasite has evolved a novel mechanism to limit the formation of CP/LP C3 convertase using a surface expressed protein called complement C2 receptor inhibitor trispanning (CRIT). CRIT contains a 27 residue N-terminal extracellular domain (ed1) which houses a specific segment of 11-amino acids (H17-Y27) termed CRIT-H17, which shares 55% identity and 73% similarity with the C4 β-chain (Inal and Schifferli, 2002). Based on its structural similarity with the C4 β-chain as revealed by antibody cross-reactivity and peptide inhibition studies, CRIT-ed1 was postulated to function as a C4-like peptide which could interact with C2 and prevent complement activation. Indeed, both ed1 and H17 CRIT peptides were observed to interact specifically with the C2a fragment. Finally, CRIT-ed1 prevented C1s mediated degradation of CRIT-ed1 bound C2 thus demonstrating its role as a decoy C2 receptor that competes with C4b for C2 and prevents cleavage of C2 by C1s. Subsequently, CRIT-H17 was reported to also interact with FB and interfere with its cleavage by FD (Hui et al., 2006). Interestingly, a human homologue of CRIT with CP inhibiting properties has also been described (Inal et al., 2005), which raises the possibility of gene transfer from host to the parasite.

3.4 Recruitment of C4BP

C4BP is a 500 kDa plasma glycoprotein and is the major soluble inhibitor of the CP/LP. It is composed of seven identical 75 kDa α-chains and one 40 kDa β-chain consisting of 8 and 3 CCP domains respectively (Figure 3A) (D. Ermert and Blom, 2016). C4BP performs its inhibitory activity through binding and controlling the function of activated C4b and C3b (Figure 3B). This inhibitory function is localised to the α-chain CCP1-3 domains which interacts electrostatically with C4b through a cluster of positively charged amino acids at the CCP1 and CCP2 interface (Blom et al., 1999). C4BP, like other soluble regulators, is highly abundant in plasma. Consequently, to survive complement destruction, microbes have evolved to recruit and use negative regulators like C4BP to combat complement.

Several microbes bind C4BP (Suppl Table 2) (reviewed in (Avirutnan et al., 2011; D. Ermert and Blom, 2016; Luo et al., 2011; Meri et al., 2004; Shayakhmetov et al., 2005; Vogl et al., 2008). Similar to binding FH, binding of C4BP inhibits complement at relatively early stages of the cascade and therefore effectively stalls complement activation prior to excessive downstream amplification. Although the binding sites on C4BP for different pathogens span nearly every CCP domain there is a strong predilection to target domains 1-2 and 7-8 (Figure 3B; Suppl Table 2).

S. pyogenes, a human specific pathogen, recruits C4BP through surface expressed M-proteins. M proteins are dimeric α-helical coiled coils which possess an extracellular hypervariable N-terminal region (HVR) (Ghosh, 2011). Despite this variability the HVR interacts exclusively with CCP1-2 of C4BP in the overwhelming majority of M-types tested (Persson et al., 2006), indicating conservation of this key function through evolution. Despite overlapping binding sites for M proteins and C4b on C4BP (Blom et al., 2000), the heptameric structure of C4BP permits *S. pyogenes* to bind the C4BP through some of its α-chain 'arms', while others are free to interact with C4b/C3b and perform cofactor and decay-accelerating activity functions. Another surface expressed member of the M-protein family, called protein H, also binds C4BP (D. Ermert et al., 2013). Protein H is expressed in approximately 30% of the highly virulent M1 strains (D. Ermert et al., 2018) and is encoded adjacent to M protein, suggesting it arose by gene duplication. Protein H binds multiple ligands including IgG (Akesson et al., 1990), and similar to protein A on *S. aureus*, binding occurs through the Fc region rendering IgG functionally effete (i.e., unable to activate complement or engage FcR). Interestingly, C4BP binding mediated through protein H was enhanced in the presence of human IgG (Hu-IgG), specifically through interaction with the Hu-IgG Fc domains. Interaction of Hu-IgG Fc with protein H results in a stable, dimeric form of protein H which translates to more C4BP binding sites (David Ermert et al., 2019). Crucially, enhanced C4BP binding mediated through protein H – Hu-IgG Fc interaction diminished complement activation, impeded bacterial killing by neutrophils and enhanced lethality of *S. pyogenes* in a murine model that incorporated Hu-IgG and human C4BP (D. Ermert et al., 2018).

Nonclassical cell surface associated proteins (also referred to as 'moonlighting proteins') are also involved in recruiting C4BP to the microbial surface. *S. pneumoniae* uses the glycolytic enzyme enolase as an additional C4BP binding protein, interacting with both CCP1/2 and CCP8

of C4BP (V. Agarwal et al., 2012). C4BP recruited to the pneumococcal surface via enolase retains its cofactor activity, promoting FI-mediated C4b degradation.

4. Preventing cleavage of C5, chemotaxis and MAC assembly

C5 convertase-mediated cleavage of C5 generates C5a, a powerful chemoattractant and C5b, the initiator of the MAC. C5a is a potent anaphylatoxin, which alerts inflammatory cells to the presence of pathogens, recruits immune cells to the site of infection and activates phagocytic cells to secrete reactive oxidants and microbicidal enzymes, all critical for innate defence (Guo and Ward, 2005). As a consequence, bacteria have developed strategies to deal with this onslaught.

S. aureus secretes a molecule, staphylococcal superantigen-like 7 (SSL-7) protein, which binds C5 with nanomolar affinity and prevents C5 interaction with either CP/LP or AP C5 convertases (Bestebroer et al., 2010; Langley et al., 2005). Additionally, SSL-7 binds avidly to monomeric IgA1 and IgA2 and blocks their interaction with FcαRI, thus disrupting FcαRI – mediated phagocytosis (Langley et al., 2005). SSL-7 repressed both phagocyte production of reactive oxygen species and phagocytosis of *S. aureus* in a human whole blood model. Interestingly, SSL-7 inhibition of C5-C5 convertase binding and phagocytosis is enhanced in the presence of IgA – it is thought that IgA may participate in steric hindrance of C5 cleavage (Bestebroer et al., 2010).

Certain major human pathogens have evolved distinct strategies to interfere with leukocyte migration to infection sites. Chemotaxis inhibitory protein of *S aureus* (CHIPS) is a secreted molecule which interrupts C5a and formylated peptide mediated neutrophil recruitment (de Haas et al., 2004). CHIPS binds avidly to both the formyl peptide and C5a transmembrane G-protein coupled receptors expressed on the neutrophil surface, diminishing chemotaxis and promoting infection. *S. pyogenes* uses two proteins to counteract C5a-dependent recruitment and activation of professional phagocytes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme, but moonlights as a complement evasin. GAPDH has been observed on the bacterial surface where it binds and sequesters C5a (Terao et al., 2006). In addition, anchored to the streptococcal cell wall is the classical C5a peptidase, ScpA, a subtilisin-like serine protease which efficiently cleaves C5a at its C-terminus to inactivate its chemotactic function (Cleary et al., 1992) and promotes bacterial

dissemination in murine models of infection (Ji et al., 1996). It is proposed that both GAPDH and C5a are necessary for efficient cleavage of surface bound C5a (Terao et al., 2006).

Only a few instances of pathogen-encoded terminal pathway inhibitors have been reported. *B. burgdorferi* possess two such surface-expressed evasins; CspA (Hallstrom et al., 2013) and a CD59-like protein (Pausa et al., 2003). Molecular analysis revealed that CspA binds both C7 and C9 in a manner similar to that of the host vitronectin (Vn) (Hallstrom et al., 2013). Although CspA binds C7 it does not interfere with interaction of C7 with C5b-6. Instead CspA binds both C7 and C9 simultaneously. Binding of C7 and C9 are localised to a 107-residue region within the CspA protein, which can inhibit $ZnCl_2$-induced C9 polymerisation. Additionally, transforming serum-sensitive *Borrelia garinii* with a plasmid-containing CspA enhanced serum resistance and blocked MAC assembly at the level of C7 (Hallstrom et al., 2013). These data suggested that CspA interferes with both MAC insertion into the plasma membrane and polymerisation at the C9 stage. Of note, CspA is also referred to as complement regulator-acquiring surface protein-1 (CRASP-1) because it binds FH (Kraiczy and Stevenson, 2013). A recent study showed that a CspA mutant that lacked the ability to bind FH but retained the capacity to bind to C7 and C9, did not protect bacteria from lysis and failed to survive in mice or ticks (Hart et al., 2018). These data suggest that inhibition of MAC formation alone by CspA, in the absence of FH binding, is insufficient for serum resistance and pathogenesis.

A unique mechanism of terminal complement component extrusion by *Salmonella minnesota* was described by Joiner *et al*, where incorporation of C8 and C9 into the MAC complex results in extrusion of the entire C5b-9 complex from the bacterial membrane (Joiner et al., 1982a; Joiner et al., 1982b).

Vn is a glycoprotein that is present in abundant amounts in plasma and numerous other tissues. The presence of Vn in diverse anatomical regions highlights its importance in many biological processes including cell migration, adhesion, tissue repair and regulation of the MAC formation (Preissner and Seiffert, 1998). Vn is composed of an N-terminal somatomedin-B domain, a cell receptor RGD binding motif, four haemopexin-like binding motifs and three heparin binding domain (HBD) (Preissner and Seiffert, 1998; Singh et al., 2010). Vn targets two distinct steps of the MAC assembly. It binds to the membrane binding site of C5b-7 and prevents its insertion into membranes (Milis et al., 1993). Second, regions localised to the HBDs of Vn bind C9 and prevent C9 polymerisation (Milis et al., 1993). Analogous to microbial

recruitment of FH and C4BP, several pathogens have evolved to acquire and localise Vn to their surface thereby inhibiting MAC formation (Suppl Table 3). *Haemophilus influenzae* type B (Hib) utilises a highly conserved non-pilus trimeric autotransporter, Haemophilus surface fibrils (Hsf) to capture Vn (Hallstrom et al., 2006). The N-terminal HBD of Hsf interacts with Vn. Hsf interacts with Vn via two distinct binding pockets Hsf $^{608\text{-}1351}$ and Hsf $^{1536\text{-}2414}$ potentially permitting one Hsf molecule to interact with two Vn molecules (Hallstrom et al., 2006). Deletion of *hsf* results in significant killing of Hib in serum bactericidal assays.

It is important to note that Vn is a key component of the extracellular matrix (ECM). Microbial interaction with exposed ECM proteins including Vn contributes to adherence, which is a prerequisite for infection. Numerous papers have highlighted bacterial interaction with Vn in the context of adherence and the reader is referred to an excellent review by Singh and colleagues (Singh et al., 2010)).

5. Proteolytic cleavage

Neutralisation of complement proteins via degradation represents another method of complement evasion. Two mechanisms result in proteolytic cleavage of complement proteins: 1) Direct, via pathogen expressed enzymes and 2) acquisition and/or activation of host plasminogen for indirect, plasmin-mediated complement degradation.

5.1 Direct attack on complement components

Bacterial proteases fall into several categories based on mechanism of action, structure and function and play essential roles in bacterial physiology and pathogenesis (Culp and Wright, 2017). Interestingly, microbial proteases from these different categories degrade complement proteins with overlapping specificity (Suppl Table 4) highlighting a strong selective pressure for protease-mediated complement degradation. Unsurprisingly, the favoured complement target is C3, which will be the focus of this section. However, proteolytic degradation of IgG, C1q, properdin, C2, C4, C5, C5a and MAC components have all been described (Suppl Table 4).

S. aureus secretes four proteases all of which target and degrade C3 and other complement proteins. One of these proteases, the zinc-dependent metalloprotease, aureolysin (Aur), targets C3 at a specific site that is only two amino acids C-terminal to the C3 convertase site, resulting in release of active C3a and C3b (Laarman et al., 2011). Under physiological conditions, Aur mediated cleavage of C3 works in conjunction with host regulators, FH and FI,

resulting in proteolytic inactivation of C3b. Crucially, secreted proteases degrade C3 in solution, away from the bacterial surface, and thus prevent C3 convertase formation and C3b deposition on bacteria. Gelatinase E protease (GelE) secreted by *E. faecalis* also cleaves C3 in a convertase-like fashion, leading to fluid-phase C3 consumption (Park et al., 2008). In contrast to Aur, GelE can also cleave surface-bound iC3b, which would limit engagement of CR3 on phagocytes. A highly efficient mechanism of C3 degradation is provided by the *S. pyogenes* cysteine protease, SpeB. SpeB is a chromosomally encoded genetically conserved virulence factor that is expressed by the vast majority of *S. pyogenes* clinical isolates (Olsen et al., 2015). Central to the complement inhibitory property of SpeB is its broad substrate specificity, permitting efficient cleavage of a large array of complement and innate immune mediators (Nelson et al., 2011). SpeB rapidly degrades the α and β chains of C3 and C3b at multiple sites (Terao et al., 2008). This rapid cleavage prevents C3b binding to the bacterial surface and impairs phagocytosis. The role of omptin proteases elaborated by Salmonella and Shigella in cleaving C3b and facilitating intracellular survival of bacteria is discussed below.

It is important to note that the substrates for bacterial proteases are not restricted to complement. Many if not all of the enzymes listed in Suppl Table 4 degrade a wide spectrum of innate immune factors such as antimicrobial peptides, chemokines, cytokines and related receptors and protease activated receptors (Potempa and Pike, 2009). However, regardless of the selective pressure driving protease evolution, their broad use by bacteria in avoiding complement detection is evident and represents a powerful mechanism of complement evasion.

5.2 Plasminogen binding/activation proteins

The host inflammatory response to infection results in the activation of multiple innate immune pathways that often 'cross-talk' to restrict, entrap and eliminate microbial pathogens. A recurring mechanism pathogens use is to manipulate the fibrinolytic system, specifically targeting plasminogen (PLG) activation (Bhattacharya et al., 2012; Potempa and Pike, 2009). PLG is a liver-derived glycoprotein present as an inactive proenzyme in human serum. The conversion of PLG to plasmin (Pm) is essential for the resolution of fibrin clots and is mediated by host activators urokinase-type plasminogen activator (uPA) or tissue-type plasminogen activator (tPA) (Bhattacharya et al., 2012). Plasmin is a serine protease with relatively low substrate specificity. In addition to its primary substrate fibrinogen, plasmin

cleaves a variety of extracellular matrix proteins and the complement components C3b and C5 (Bhattacharya et al., 2012). Further, PLG itself serves as a complement inhibitor; in the presence of FH, PLG enhances FI mediated C3b inactivation (Barthel et al., 2012). Therefore, hijacking the proteolytic activity of plasmin(ogen) benefits the pathogen and is achieved either by 1) recruiting plasminogen to the bacterial surface, which becomes activated by host plasminogen activators (Figure 4, right side) or 2) expression of bacterial proteins which cleaves PLG to the active form, Pm (Figure 4, left side). *Acinetobacter baumannii* is a Gram-negative, multidrug-resistant and complement-resistant human pathogen. A recent study showed that *A. baumannii* recruits PLG using translation elongation factor Tuf, whereby host uPA then cleaves surface bound PLG to Pm, which in turn cleaves C3b (Koenigs et al., 2015). This works adds to the growing list of glycolytic and metabolic enzymes and chaperones with moonlighting activities that play important roles in complement evasion and virulence.

Bacteria-derived PLG activators that work in a similar fashion to host plasminogen activators may also aid in usurping PLG. These proteins have been described thus far only in gram-negative pathogens and belong to a family of outer membrane aspartyl proteases known as Omptins (Suppl Table 4). *Salmonella enterica* expresses one such protease, PgtE, which modulates Pm activity by both processing PLG and inhibiting the Pm inhibitor, α_2-antiplasmin (Lahteenmaki et al., 2005). Although PgtE can cleave purified complement proteins (C3b, C4b and C5), enhanced cleavage is observed in the presence of PLG (Ramu et al., 2007), underscoring the anti-complement activity performed by plasmin(ogen) hijacking.

6. Intracellular pathogens and complement

The intracellular environment has classically been considered a safe haven for pathogens from detection by host complement. During their journey to gain intracellular access, pathogens must survive the extracellular milieu where they encounter antibodies and complement. Well-characterised intracellular pathogen detection techniques typically rely on the recognition of PAMPs by Toll-like receptors and nucleic acid receptors located in the cytosol (Kawai and Akira, 2008). However, accumulating evidence indicates that antibodies and complement components deposited on pathogens are carried inside the host cell upon invasion / internalisation, triggering antimicrobial pathways and thus representing a novel method of immune surveillance (Figure 5) (McEwan et al., 2013; Sorbara et al., 2018; Tam et al., 2014).

The role of complement in promoting autophagy-mediated restriction of pathogens in non-immune cells was recently examined. C3 – specifically the C3d domain – interacts with ATG16L1, a cytosolic protein essential for organisation of the autophagy machinery (Sorbara et al., 2018). Opsonisation of the intracellular pathogens, *Listeria monocytogenes* and *Shigella flexneri* with C5-depleted human serum prior to incubation with cells resulted in enhanced targeting of bacteria by autophagy proteins ATG16L1 or LC3 compared to bacteria not coated with C3. Furthermore, growth restriction of *L. monocytogenes* was enhanced in a C3-dependent fashion and was reversed in ATG16L1 deficient cells. *In vivo*, C3-deficient mice had a higher *L. monocytogenes* mucosal burden using an intra-gastric infection model compared to wild-type mice. Importantly, treatment of C3-deficient mice with rapamycin, an inducer of autophagy, accelerated bacterial killing. Taken together these results indicate that pathogen-bound C3 associates with ATG16L1 to promote autophagy-dependent restriction of *L. monocytogenes*. Unsurprisingly, certain pathogens have thwarted C3-mediated autophagy restriction. Two intracellular pathogens, *S. flexneri* and *S. typhimurium,* utilise surface expressed omptin proteases to rapidly cleave C3 to limit autophagy and promote intracellular survival (Figure 5A) (Sorbara et al., 2018).

Intracellular sensing of C3 deposited on human viruses induces immune signalling and activation of degradation pathways independent of autophagy (Tam et al., 2014). Infection of human embryonic kidney (HEK) 293T cells with non-enveloped viruses stimulated nuclear factor κB (NF-κB) expression only when the infecting virus was pre-opsonised with serum. C3 mediated NF-κB and subsequent pro-inflammatory cytokine production was dependent on C3-coated viral particles reaching the cytosol, suggesting that C3 functioned as a damage-associated molecular pattern (DAMP) to stimulate innate immune responses (Figure 5B). Critically, inhibition of mitochondrial antiviral signalling (MAVS) disrupted C3-mediated immune activation. MAVS induction leads to the reorganisation of downstream molecules that culminates in dimerization and activation of interferon regulatory factor 3 (IRF3) and expression of antiviral interferons (Seth et al., 2005). In addition, C3 labelling of virions activated intracellular valosin-containing protein (VCP) and proteasome dependent pathways restricting viral infection (Tam et al., 2014).

The evolution of pathogen-specific counter measures to mitigate intracellular complement driven viral restriction underlines the importance of this antiviral response. Human rhinoviruses (HRVs), the most common cause of upper respiratory tract infections, employ a

cystolic 3C protease predicted to impair C3 mediated intracellular immunity. Recombinant HRV 3C protease cleaves C3 specifically deposited on viral particles (Tam et al., 2014). Expression of HRV 3C protease within HEK 293T cells prior to C3 opsonised viral infection significantly reduced NF-κB expression. Additionally, infection of HEK 293T cells with serum opsonised HRV resulted in rapid cleavage of intracellular C3. In contrast, serum opsonised adenovirus (AdV), which does not express 3C proteases, left C3 intact and rendered the C3-coated virus susceptible to intracellular sensing.

Importantly, intracellular detection of humoral components is not restricted to complement. Intracellular immune responses are also activated following intracellular sensing of antibody-coated pathogens by the IgG receptor, tripartite motif-containing 21 (TRIM21) (Mallery et al., 2010). Antibody-coated AdV is rapidly bound by TRIM21 which specifically recognises the Fc domain. TRIM21 displays E3 ubiquitin ligase activity and targets the virus for degradation via the proteasomal pathway (Mallery et al., 2010). Certain pathogens employ proteases which can degrade IgG or bind IgG via the Fc portion masking recognition. It is tempting to speculate whether these evasion mechanisms are also involved in subverting Ab mediated intracellular immunity.

7. Exploitation of complement facilitates microbial entry of host cells

Complement receptors and regulators decorate a diverse range of immune and non-immune cells and are fundamental in mediating immune complex clearance, phagocytosis and complement regulation (Holers, 2014; Merle et al., 2015; Noris and Remuzzi, 2013). Pathogens have evolved to hijack these abundant cell surface proteins, namely complement receptors, CD35/CR1, CD21/CR2 and CD11b/CD18/CR3 and complement regulators CD55/DAF and CD46/MCP, in order to enter host cells, escaping immune detection and enhancing survival.

An excellent example of microbial manipulation of complement receptors is highlighted by *Plasmodium falciparum*, the causative agent of malaria. *P. falciparum* is an obligate intracellular parasite, which survives within the human host by invading erythrocytes in a complex, multistep process (Schmidt et al., 2015). Central to invasion is the expression of parasite reticulocyte-binding like proteins, one of which, PfRh4, directly targets CR1 (Tham et al., 2010). PfRh4 specifically binds the N -terminal CCP1-3 region of CR1 (Tham et al., 2011), normally reserved for C3b/C4b binding and accelerating decay of both CP and AP C3 and C5 convertases (Holers, 2014). Parasite binding of CR1 did not affect C3b/C4b binding nor

cofactor activity but did inhibit decay accelerating activity. Parasitic invasion of erythrocytes occurs rapidly, with a transient interaction between parasite and CR1, suggesting a minimal impact on complement regulation. Instead, by targeting an essential region on CR1, the parasite may take advantage of a highly conserved structure as a means of entry (Tham et al., 2011).

Other obligate intracellular pathogens have evolved a non-specialised approach, permitting deposition of complement fragments and relying on this opsonisation as a means of promoting host cell entry. Here interaction of covalently attached C3 activation products with CR1/CR3 facilitate pathogen entry, as described for important human pathogens, *Mycobacterium tuberculosis* (Schorey et al., 1997), *Leishmania spp* (Da Silva et al., 1989) and human immunodeficiency virus (Bajtay et al., 2004). The reader is directed to excellent reviews which provide an in-depth analysis of the pathogenic exploitation of complement receptors and regulators (Cattaneo, 2004; Fernandez et al., 2019; Lindahl et al., 2000).

8. Discussion and outlook

The success of pathogens requires an ability to colonise their hosts, extract nutrients to proliferate and dampen or resist immune responses associated with their removal (Figure 6A). The importance of complement evasion for microbial pathogenicity is evident from the numerous, independently evolved strategies outlined in this review, indicating that this is a conserved requirement for infection (Figure 6B).

Technological advances have made it possible to examine and unravel the biochemical and structural features governing microbial complement inhibition. The next essential step is to use this information to develop therapeutic avenues to disrupt these evasive mechanisms. Understanding the role of individual evasins during infection will facilitate the rational design of therapeutic intervention strategies. These could be based on a number of approaches including the development of monoclonal neutralising antibodies raised against specific evasins and small molecule inhibitors designed to disrupt evasin function. Microbial proteins that bind complement inhibitors may prove to be effective antigens for vaccines; meningococcal factor H binding protein (FHbp) is one such example (Perez et al., 2018; Rappuoli et al., 2018). Elucidating the basis of human FH-FHbp interactions proved useful in designing FHbp molecules that did not bind human FH, which further augmented bactericidal antibody responses (Beernink et al., 2011; Granoff et al., 2016). Such a strategy could be

employed to design vaccines that incorporate microbial proteins that bind human complement inhibitors in order to cripple critical pathogen immune evasion mechanisms. In addition, fusion proteins designed to interfere with essential evasion mechanisms are being developed which efficiently re-sensitizes bacteria to complement (Blom et al., 2017; Ram et al., 2016). Alternatively, augmenting immune responses by enhancing the immunogenicity of target antigens is being explored. Here bacterial complement activators are used as molecular adjuvants opsonising antigens, facilitating increased humoral immune responses (Yang et al., 2018). These approaches offer novel methods for controlling infection and help address the problem of antimicrobial resistance that threatens human health globally.

Bacterial whole genome sequencing has revolutionised our understanding of pathogen biology (Didelot et al., 2012; Laabei et al., 2014). The abundant genomic data can be used to mine and characterise novel complement evasins. Alternatively, this genetic data has the potential to be used in functional genomic approaches (Laabei and Massey, 2016) aimed at unravelling how complement evasins are regulated at the genetic level, offering more targets for intervention and providing a greater understanding of pathogen virulence.

The role of complement as solely an extracellular feature in pathogen immune surveillance has been challenged. Intracellular recognition of C3 labelled bacteria and viruses results in the activation of signalling and degradative pathways and offer an insight into how host cells deal with microbial invasion. In addition to a novel intracellular recycling pathway for C3 (Elvington et al., 2017), a new 'form' of C3 can be transcribed from an alternative start codon that results in C3 being retained in the cytosol (King et al., 2019); these data have firmly established C3 as a major player in intracellular processes (Hess and Kemper, 2016; Liszewski et al., 2013). What role does intracellular C3 play in sensing pathogens, what other ligands are required for activation of immune signalling cascades and have pathogens evolved mechanisms to circumvent these systems within the harsh intracellular environment? At the genetic level, what are the microbial regulatory elements governing complement evasion? Are complement evasins constitutively expressed or induced under specific microenvironmental or stressful conditions? Moreover, in relation to the apparent redundancy of complement evasins observed in certain pathogens, is the expression of subsets of complement evasins infection specific?

There is a lot to learn about how pathogens and complement interact and a more intensive scrutiny of the above questions may provide therapeutic targets to universally repress

764 evasion. Disruption of essential microbial complement evasive strategies will give our immune
765 system a significant boost in fighting infection and impose less selective pressure for the
766 development of resistance than conventional antimicrobial approaches.
767
768 **Acknowledgements**
769 M.L. was supported by the Lars Hierta Memorial Foundation, the Tore Nilssons Foundation,
770 the Royal Physiographic Society of Lund and an internal University of Bath grant. D.E. was
771 supported by the Gyllenstiernska Krapperup Foundation. S.R. is supported by National
772 Institutes of Health grants AI114790, AI132296, AI136007, AI119327 and AI141181.
773
774
775 **References**
776
777 Agarwal, S., Vasudhev, S., DeOliveira, R.B., Ram, S., 2014. Inhibition of the classical pathway
778 of complement by meningococcal capsular polysaccharides. J Immunol 193, 1855-1863.
779 Agarwal, V., Hammerschmidt, S., Malm, S., Bergmann, S., Riesbeck, K., Blom, A.M., 2012.
780 Enolase of Streptococcus pneumoniae binds human complement inhibitor C4b-binding
781 protein and contributes to complement evasion. J Immunol 189, 3575-3584.
782 Akesson, P., Cooney, J., Kishimoto, F., Bjorck, L., 1990. Protein H--a novel IgG binding
783 bacterial protein. Mol Immunol 27, 523-531.
784 Avirutnan, P., Fuchs, A., Hauhart, R.E., Somnuke, P., Youn, S., Diamond, M.S., Atkinson, J.P.,
785 2010. Antagonism of the complement component C4 by flavivirus nonstructural protein NS1.
786 J Exp Med 207, 793-806.
787 Avirutnan, P., Hauhart, R.E., Somnuke, P., Blom, A.M., Diamond, M.S., Atkinson, J.P., 2011.
788 Binding of flavivirus nonstructural protein NS1 to C4b binding protein modulates
789 complement activation. J Immunol 187, 424-433.
790 Bajtay, Z., Speth, C., Erdei, A., Dierich, M.P., 2004. Cutting edge: productive HIV-1 infection of
791 dendritic cells via complement receptor type 3 (CR3, CD11b/CD18). J Immunol 173, 4775-
792 4778.
793 Barilla-LaBarca, M.L., Atkinson, J.P., 2003. Rheumatic syndromes associated with
794 complement deficiency. Curr Opin Rheumatol 15, 55-60.
795 Barthel, D., Schindler, S., Zipfel, P.F., 2012. Plasminogen is a complement inhibitor. J Biol
796 Chem 287, 18831-18842.
797 Bayly-Jones, C., Bubeck, D., Dunstone, M.A., 2017. The mystery behind membrane insertion:
798 a review of the complement membrane attack complex. Philos Trans R Soc Lond B Biol Sci
799 372.
800 Beernink, P.T., Shaughnessy, J., Braga, E.M., Liu, Q., Rice, P.A., Ram, S., Granoff, D.M., 2011.
801 A meningococcal factor H binding protein mutant that eliminates factor H binding enhances
802 protective antibody responses to vaccination. J Immunol 186, 3606-3614.
803 Berends, E.T., Kuipers, A., Ravesloot, M.M., Urbanus, R.T., Rooijakkers, S.H., 2014. Bacteria
804 under stress by complement and coagulation. FEMS Microbiol Rev 38, 1146-1171.

Bestebroer, J., Aerts, P.C., Rooijakkers, S.H., Pandey, M.K., Kohl, J., van Strijp, J.A., de Haas, C.J., 2010. Functional basis for complement evasion by staphylococcal superantigen-like 7. Cell Microbiol 12, 1506-1516.
Bexborn, F., Andersson, P.O., Chen, H., Nilsson, B., Ekdahl, K.N., 2008. The tick-over theory revisited: formation and regulation of the soluble alternative complement C3 convertase (C3(H2O)Bb). Mol Immunol 45, 2370-2379.
Bhattacharya, S., Ploplis, V.A., Castellino, F.J., 2012. Bacterial plasminogen receptors utilize host plasminogen system for effective invasion and dissemination. J Biomed Biotechnol 2012, 482096.
Biesma, D.H., Hannema, A.J., van Velzen-Blad, H., Mulder, L., van Zwieten, R., Kluijt, I., Roos, D., 2001. A family with complement factor D deficiency. J Clin Invest 108, 233-240.
Blom, A.M., Berggard, K., Webb, J.H., Lindahl, G., Villoutreix, B.O., Dahlback, B., 2000. Human C4b-binding protein has overlapping, but not identical, binding sites for C4b and streptococcal M proteins. J Immunol 164, 5328-5336.
Blom, A.M., Magda, M., Kohl, L., Shaughnessy, J., Lambris, J.D., Ram, S., Ermert, D., 2017. Factor H-IgG Chimeric Proteins as a Therapeutic Approach against the Gram-Positive Bacterial Pathogen Streptococcus pyogenes. J Immunol 199, 3828-3839.
Blom, A.M., Webb, J., Villoutreix, B.O., Dahlback, B., 1999. A cluster of positively charged amino acids in the C4BP alpha-chain is crucial for C4b binding and factor I cofactor function. J Biol Chem 274, 19237-19245.
Brandtzaeg, P., Mollnes, T.E., Kierulf, P., 1989. Complement activation and endotoxin levels in systemic meningococcal disease. J Infect Dis 160, 58-65.
Caesar, J.J., Lavender, H., Ward, P.N., Exley, R.M., Eaton, J., Chittock, E., Malik, T.H., Goiecoechea De Jorge, E., Pickering, M.C., Tang, C.M., Lea, S.M., 2014. Competition between antagonistic complement factors for a single protein on N. meningitidis rules disease susceptibility. Elife 3.
Cattaneo, R., 2004. Four viruses, two bacteria, and one receptor: membrane cofactor protein (CD46) as pathogens' magnet. J Virol 78, 4385-4388.
Cleary, P.P., Prahbu, U., Dale, J.B., Wexler, D.E., Handley, J., 1992. Streptococcal C5a peptidase is a highly specific endopeptidase. Infect Immun 60, 5219-5223.
Crew, P.E., Abara, W.E., McCulley, L., Waldron, P.E., Kirkcaldy, R.D., Weston, E.J., Bernstein, K.T., Jones, S.C., Bersoff-Matcha, S.J., 2018. Disseminated gonococcal infections in patients receiving eculizumab: a case series. Clin Infect Dis.
Crew, P.E., McNamara, L., Waldron, P.E., McCulley, L., Jones, S.C., Bersoff-Matcha, S.J., 2019. Unusual Neisseria species as a cause of infection in patients taking eculizumab. J Infect 78, 113-118.
Culp, E., Wright, G.D., 2017. Bacterial proteases, untapped antimicrobial drug targets. J Antibiot (Tokyo) 70, 366-377.
Da Silva, R.P., Hall, B.F., Joiner, K.A., Sacks, D.L., 1989. CR1, the C3b receptor, mediates binding of infective Leishmania major metacyclic promastigotes to human macrophages. J Immunol 143, 617-622.
Davis, A.E., 3rd, Lu, F., Mejia, P., 2010. C1 inhibitor, a multi-functional serine protease inhibitor. Thromb Haemost 104, 886-893.
de Haas, C.J., Veldkamp, K.E., Peschel, A., Weerkamp, F., Van Wamel, W.J., Heezius, E.C., Poppelier, M.J., Van Kessel, K.P., van Strijp, J.A., 2004. Chemotaxis inhibitory protein of Staphylococcus aureus, a bacterial antiinflammatory agent. J Exp Med 199, 687-695.

de Vries, S.P., Bootsma, H.J., Hays, J.P., Hermans, P.W., 2009. Molecular aspects of Moraxella catarrhalis pathogenesis. Microbiol Mol Biol Rev 73, 389-406, Table of Contents.
DeDent, A.C., McAdow, M., Schneewind, O., 2007. Distribution of protein A on the surface of Staphylococcus aureus. J Bacteriol 189, 4473-4484.
Devyatyarova-Johnson, M., Rees, I.H., Robertson, B.D., Turner, M.W., Klein, N.J., Jack, D.L., 2000. The lipopolysaccharide structures of Salmonella enterica serovar Typhimurium and Neisseria gonorrhoeae determine the attachment of human mannose-binding lectin to intact organisms. Infect Immun 68, 3894-3899.
Didelot, X., Eyre, D.W., Cule, M., Ip, C.L., Ansari, M.A., Griffiths, D., Vaughan, A., O'Connor, L., Golubchik, T., Batty, E.M., Piazza, P., Wilson, D.J., Bowden, R., Donnelly, P.J., Dingle, K.E., Wilcox, M., Walker, A.S., Crook, D.W., Peto, T.E., Harding, R.M., 2012. Microevolutionary analysis of Clostridium difficile genomes to investigate transmission. Genome Biol 13, R118.
Diebolder, C.A., Beurskens, F.J., de Jong, R.N., Koning, R.I., Strumane, K., Lindorfer, M.A., Voorhorst, M., Ugurlar, D., Rosati, S., Heck, A.J., van de Winkel, J.G., Wilson, I.A., Koster, A.J., Taylor, R.P., Saphire, E.O., Burton, D.R., Schuurman, J., Gros, P., Parren, P.W., 2014. Complement is activated by IgG hexamers assembled at the cell surface. Science 343, 1260-1263.
Dunlop, L.R., Oehlberg, K.A., Reid, J.J., Avci, D., Rosengard, A.M., 2003. Variola virus immune evasion proteins. Microbes Infect 5, 1049-1056.
Elkins, C., Carbonetti, N.H., Varela, V.A., Stirewalt, D., Klapper, D.G., Sparling, P.F., 1992. Antibodies to N-terminal peptides of gonococcal porin are bactericidal when gonococcal lipopolysaccharide is not sialylated. Mol Microbiol 6, 2617-2628.
Elvington, M., Liszewski, M.K., Atkinson, J.P., 2016. Evolution of the complement system: from defense of the single cell to guardian of the intravascular space. Immunol Rev 274, 9-15.
Elvington, M., Liszewski, M.K., Bertram, P., Kulkarni, H.S., Atkinson, J.P., 2017. A C3(H20) recycling pathway is a component of the intracellular complement system. J Clin Invest 127, 970-981.
Ermert, D., Blom, A.M., 2016. C4b-binding protein: The good, the bad and the deadly. Novel functions of an old friend. Immunol Lett 169, 82-92.
Ermert, D., Laabei, M., Weckel, A., Mörgelin, M., Lundqvist, M., Björck, L., Ram, S., Linse, S., Blom, A.M., 2019. The Molecular Basis of Human IgG-Mediated Enhancement of C4b-Binding Protein Recruitment to Group A Streptococcus. Frontiers in Immunology 10.
Ermert, D., Shaughnessy, J., Joeris, T., Kaplan, J., Pang, C.J., Kurt-Jones, E.A., Rice, P.A., Ram, S., Blom, A.M., 2015. Virulence of Group A Streptococci Is Enhanced by Human Complement Inhibitors. PLoS Pathog 11, e1005043.
Ermert, D., Weckel, A., Agarwal, V., Frick, I.M., Bjorck, L., Blom, A.M., 2013. Binding of complement inhibitor C4b-binding protein to a highly virulent Streptococcus pyogenes M1 strain is mediated by protein H and enhances adhesion to and invasion of endothelial cells. J Biol Chem 288, 32172-32183.
Ermert, D., Weckel, A., Magda, M., Morgelin, M., Shaughnessy, J., Rice, P.A., Bjorck, L., Ram, S., Blom, A.M., 2018. Human IgG Increases Virulence of Streptococcus pyogenes through Complement Evasion. J Immunol 200, 3495-3505.
Fernandez, F.J., Gomez, S., Vega, M.C., 2019. Pathogens' toolbox to manipulate human complement. Semin Cell Dev Biol 85, 98-109.
Ferreira, V.P., Pangburn, M.K., Cortes, C., 2010. Complement control protein factor H: the good, the bad, and the inadequate. Mol Immunol 47, 2187-2197.

898 Figueroa, J., Andreoni, J., Densen, P., 1993. Complement deficiency states and
899 meningococcal disease. Immunol Res 12, 295-311.
900 Figueroa, J.E., Densen, P., 1991. Infectious diseases associated with complement
901 deficiencies. Clin Microbiol Rev 4, 359-395.
902 Fijen, C.A., Kuijper, E.J., te Bulte, M.T., Daha, M.R., Dankert, J., 1999a. Assessment of
903 complement deficiency in patients with meningococcal disease in The Netherlands. Clin
904 Infect Dis 28, 98-105.
905 Fijen, C.A., van den Bogaard, R., Schipper, M., Mannens, M., Schlesinger, M., Nordin, F.G.,
906 Dankert, J., Daha, M.R., Sjoholm, A.G., Truedsson, L., Kuijper, E.J., 1999b. Properdin
907 deficiency: molecular basis and disease association. Mol Immunol 36, 863-867.
908 Foster, T.J., Geoghegan, J.A., Ganesh, V.K., Hook, M., 2014. Adhesion, invasion and evasion:
909 the many functions of the surface proteins of Staphylococcus aureus. Nat Rev Microbiol 12,
910 49-62.
911 Garcia, B.L., Zhi, H., Wager, B., Hook, M., Skare, J.T., 2016. Borrelia burgdorferi BBK32
912 Inhibits the Classical Pathway by Blocking Activation of the C1 Complement Complex. PLoS
913 Pathog 12, e1005404.
914 Garred, P., Genster, N., Pilely, K., Bayarri-Olmos, R., Rosbjerg, A., Ma, Y.J., Skjoedt, M.O.,
915 2016. A journey through the lectin pathway of complement-MBL and beyond. Immunol Rev
916 274, 74-97.
917 Ghosh, P., 2011. The nonideal coiled coil of M protein and its multifarious functions in
918 pathogenesis. Adv Exp Med Biol 715, 197-211.
919 Gilbert, M., Watson, D.C., Cunningham, A.M., Jennings, M.P., Young, N.M., Wakarchuk,
920 W.W., 1996. Cloning of the lipooligosaccharide alpha-2,3-sialyltransferase from the bacterial
921 pathogens Neisseria meningitidis and Neisseria gonorrhoeae. J Biol Chem 271, 28271-28276.
922 Gordon, D.L., Kaufman, R.M., Blackmore, T.K., Kwong, J., Lublin, D.M., 1995. Identification of
923 complement regulatory domains in human factor H. J Immunol 155, 348-356.
924 Granoff, D.M., Giuntini, S., Gowans, F.A., Lujan, E., Sharkey, K., Beernink, P.T., 2016.
925 Enhanced protective antibody to a mutant meningococcal factor H-binding protein with low-
926 factor H binding. JCI Insight 1, e88907.
927 Granoff, D.M., Welsch, J.A., Ram, S., 2009. Binding of complement factor H (fH) to Neisseria
928 meningitidis is specific for human fH and inhibits complement activation by rat and rabbit
929 sera. Infect Immun 77, 764-769.
930 Gulati, S., Sastry, K., Jensenius, J.C., Rice, P.A., Ram, S., 2002. Regulation of the mannan-
931 binding lectin pathway of complement on Neisseria gonorrhoeae by C1-inhibitor and alpha
932 2-macroglobulin. J Immunol 168, 4078-4086.
933 Gulati, S., Schoenhofen, I.C., Whitfield, D.M., Cox, A.D., Li, J., St Michael, F., Vinogradov, E.V.,
934 Stupak, J., Zheng, B., Ohnishi, M., Unemo, M., Lewis, L.A., Taylor, R.E., Landig, C.S., Diaz, S.,
935 Reed, G.W., Varki, A., Rice, P.A., Ram, S., 2015. Utilizing CMP-Sialic Acid Analogs to Unravel
936 Neisseria gonorrhoeae Lipooligosaccharide-Mediated Complement Resistance and Design
937 Novel Therapeutics. PLoS Pathog 11, e1005290.
938 Guo, R.F., Ward, P.A., 2005. Role of C5a in inflammatory responses. Annu Rev Immunol 23,
939 821-852.
940 Hair, P.S., Ward, M.D., Semmes, O.J., Foster, T.J., Cunnion, K.M., 2008. Staphylococcus
941 aureus clumping factor A binds to complement regulator factor I and increases factor I
942 cleavage of C3b. J Infect Dis 198, 125-133.
943 Hallstrom, T., Siegel, C., Morgelin, M., Kraiczy, P., Skerka, C., Zipfel, P.F., 2013. CspA from
944 Borrelia burgdorferi inhibits the terminal complement pathway. MBio 4.

945 Hallstrom, T., Trajkovska, E., Forsgren, A., Riesbeck, K., 2006. Haemophilus influenzae
946 surface fibrils contribute to serum resistance by interacting with vitronectin. J Immunol 177,
947 430-436.
948 Hammel, M., Sfyroera, G., Pyrpassopoulos, S., Ricklin, D., Ramyar, K.X., Pop, M., Jin, Z.,
949 Lambris, J.D., Geisbrecht, B.V., 2007a. Characterization of Ehp, a secreted complement
950 inhibitory protein from Staphylococcus aureus. J Biol Chem 282, 30051-30061.
951 Hammel, M., Sfyroera, G., Ricklin, D., Magotti, P., Lambris, J.D., Geisbrecht, B.V., 2007b. A
952 structural basis for complement inhibition by Staphylococcus aureus. Nat Immunol 8, 430-
953 437.
954 Harboe, M., Mollnes, T.E., 2008. The alternative complement pathway revisited. J Cell Mol
955 Med 12, 1074-1084.
956 Harris, S.L., Frank, I., Yee, A., Cohen, G.H., Eisenberg, R.J., Friedman, H.M., 1990.
957 Glycoprotein C of herpes simplex virus type 1 prevents complement-mediated cell lysis and
958 virus neutralization. J Infect Dis 162, 331-337.
959 Hart, T., Nguyen, N.T.T., Nowak, N.A., Zhang, F., Linhardt, R.J., Diuk-Wasser, M., Ram, S.,
960 Kraiczy, P., Lin, Y.P., 2018. Polymorphic factor H-binding activity of CspA protects Lyme
961 borreliae from the host complement in feeding ticks to facilitate tick-to-host transmission.
962 PLoS Pathog 14, e1007106.
963 Heesterbeek, D.A., Bardoel, B.W., Parsons, E.S., Bennett, I., Ruyken, M., Doorduijn, D.J.,
964 Gorham, R.D., Jr., Berends, E.T., Pyne, A.L., Hoogenboom, B.W., Rooijakkers, S.H., 2019.
965 Bacterial killing by complement requires membrane attack complex formation via surface-
966 bound C5 convertases. EMBO J 38.
967 Hess, C., Kemper, C., 2016. Complement-Mediated Regulation of Metabolism and Basic
968 Cellular Processes. Immunity 45, 240-254.
969 Holers, V.M., 2014. Complement and its receptors: new insights into human disease. Annu
970 Rev Immunol 32, 433-459.
971 Hourcade, D.E., 2006. The role of properdin in the assembly of the alternative pathway C3
972 convertases of complement. J Biol Chem 281, 2128-2132.
973 Hovingh, E.S., van den Broek, B., Kuipers, B., Pinelli, E., Rooijakkers, S.H.M., Jongerius, I.,
974 2017. Acquisition of C1 inhibitor by Bordetella pertussis virulence associated gene 8 results
975 in C2 and C4 consumption away from the bacterial surface. PLoS Pathog 13, e1006531.
976 Huang, Y., Qiao, F., Abagyan, R., Hazard, S., Tomlinson, S., 2006. Defining the CD59-C9
977 binding interaction. J Biol Chem 281, 27398-27404.
978 Hui, K.M., Magnadottir, B., Schifferli, J.A., Inal, J.M., 2006. CRIT peptide interacts with factor
979 B and interferes with alternative pathway activation. Biochem Biophys Res Commun 344,
980 308-314.
981 Hussain, M., Becker, K., von Eiff, C., Peters, G., Herrmann, M., 2001. Analogs of Eap protein
982 are conserved and prevalent in clinical Staphylococcus aureus isolates. Clin Diagn Lab
983 Immunol 8, 1271-1276.
984 Inal, J.M., Hui, K.M., Miot, S., Lange, S., Ramirez, M.I., Schneider, B., Krueger, G., Schifferli,
985 J.A., 2005. Complement C2 receptor inhibitor trispanning: a novel human complement
986 inhibitory receptor. J Immunol 174, 356-366.
987 Inal, J.M., Schifferli, J.A., 2002. Complement C2 receptor inhibitor trispanning and the beta-
988 chain of C4 share a binding site for complement C2. J Immunol 168, 5213-5221.
989 Ji, Y., McLandsborough, L., Kondagunta, A., Cleary, P.P., 1996. C5a peptidase alters clearance
990 and trafficking of group A streptococci by infected mice. Infect Immun 64, 503-510.

Johnson, J.B., Borisevich, V., Rockx, B., Parks, G.D., 2015. A novel factor I activity in Nipah virus inhibits human complement pathways through cleavage of C3b. J Virol 89, 989-998.
Joiner, K.A., Hammer, C.H., Brown, E.J., Cole, R.J., Frank, M.M., 1982a. Studies on the mechanism of bacterial resistance to complement-mediated killing. I. Terminal complement components are deposited and released from Salmonella minnesota S218 without causing bacterial death. J Exp Med 155, 797-808.
Joiner, K.A., Hammer, C.H., Brown, E.J., Frank, M.M., 1982b. Studies on the mechanism of bacterial resistance to complement-mediated killing. II. C8 and C9 release C5b67 from the surface of Salmonella minnesota S218 because the terminal complex does not insert into the bacterial outer membrane. J Exp Med 155, 809-819.
Jongerius, I., Garcia, B.L., Geisbrecht, B.V., van Strijp, J.A., Rooijakkers, S.H., 2010. Convertase inhibitory properties of Staphylococcal extracellular complement-binding protein. J Biol Chem 285, 14973-14979.
Jongerius, I., Kohl, J., Pandey, M.K., Ruyken, M., van Kessel, K.P., van Strijp, J.A., Rooijakkers, S.H., 2007. Staphylococcal complement evasion by various convertase-blocking molecules. J Exp Med 204, 2461-2471.
Jozsi, M., 2017. Factor H Family Proteins in Complement Evasion of Microorganisms. Front Immunol 8, 571.
Kang, M., Ko, Y.P., Liang, X., Ross, C.L., Liu, Q., Murray, B.E., Hook, M., 2013. Collagen-binding microbial surface components recognizing adhesive matrix molecule (MSCRAMM) of Gram-positive bacteria inhibit complement activation via the classical pathway. J Biol Chem 288, 20520-20531.
Kawai, T., Akira, S., 2008. Toll-like receptor and RIG-I-like receptor signaling. Ann N Y Acad Sci 1143, 1-20.
Kemper, C., Hourcade, D.E., 2008. Properdin: New roles in pattern recognition and target clearance. Mol Immunol 45, 4048-4056.
Killick, J., Morisse, G., Sieger, D., Astier, A.L., 2018. Complement as a regulator of adaptive immunity. Semin Immunopathol 40, 37-48.
King, B.C., Kulak, K., Krus, U., Rosberg, R., Golec, E., Wozniak, K., Gomez, M.F., Zhang, E., O'Connell, D.J., Renstrom, E., Blom, A.M., 2019. Complement Component C3 Is Highly Expressed in Human Pancreatic Islets and Prevents beta Cell Death via ATG16L1 Interaction and Autophagy Regulation. Cell Metab 29, 202-210 e206.
Koenigs, A., Zipfel, P.F., Kraiczy, P., 2015. Translation Elongation Factor Tuf of Acinetobacter baumannii Is a Plasminogen-Binding Protein. PLoS One 10, e0134418.
Kostavasili, I., Sahu, A., Friedman, H.M., Eisenberg, R.J., Cohen, G.H., Lambris, J.D., 1997. Mechanism of complement inactivation by glycoprotein C of herpes simplex virus. J Immunol 158, 1763-1771.
Kotwal, G.J., 2000. Poxviral mimicry of complement and chemokine system components: what's the end game? Immunol Today 21, 242-248.
Kraiczy, P., Stevenson, B., 2013. Complement regulator-acquiring surface proteins of Borrelia burgdorferi: Structure, function and regulation of gene expression. Ticks Tick Borne Dis 4, 26-34.
Laabei, M., Ermert, D., 2019. Catch Me if You Can: Streptococcus pyogenes Complement Evasion Strategies. J Innate Immun 11, 3-12.
Laabei, M., Massey, R., 2016. Using functional genomics to decipher the complexity of microbial pathogenicity. Curr Genet 62, 523-525.

1037 Laabei, M., Recker, M., Rudkin, J.K., Aldeljawi, M., Gulay, Z., Sloan, T.J., Williams, P., Endres,
1038 J.L., Bayles, K.W., Fey, P.D., Yajjala, V.K., Widhelm, T., Hawkins, E., Lewis, K., Parfett, S.,
1039 Scowen, L., Peacock, S.J., Holden, M., Wilson, D., Read, T.D., van den Elsen, J., Priest, N.K.,
1040 Feil, E.J., Hurst, L.D., Josefsson, E., Massey, R.C., 2014. Predicting the virulence of MRSA from
1041 its genome sequence. Genome Res 24, 839-849.
1042 Laarman, A.J., Ruyken, M., Malone, C.L., van Strijp, J.A., Horswill, A.R., Rooijakkers, S.H.,
1043 2011. Staphylococcus aureus metalloprotease aureolysin cleaves complement C3 to mediate
1044 immune evasion. J Immunol 186, 6445-6453.
1045 Lachmann, P.J., 2009. The amplification loop of the complement pathways. Adv Immunol
1046 104, 115-149.
1047 Lahteenmaki, K., Kyllonen, P., Partanen, L., Korhonen, T.K., 2005. Antiprotease inactivation
1048 by Salmonella enterica released from infected macrophages. Cell Microbiol 7, 529-538.
1049 Lambris, J.D., Ricklin, D., Geisbrecht, B.V., 2008. Complement evasion by human pathogens.
1050 Nat Rev Microbiol 6, 132-142.
1051 Langereis, J.D., de Jonge, M.I., Weiser, J.N., 2014. Binding of human factor H to outer
1052 membrane protein P5 of non-typeable Haemophilus influenzae contributes to complement
1053 resistance. Mol Microbiol 94, 89-106.
1054 Langley, R., Wines, B., Willoughby, N., Basu, I., Proft, T., Fraser, J.D., 2005. The
1055 staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc
1056 alpha RI binding and serum killing of bacteria. J Immunol 174, 2926-2933.
1057 Law, S.K., Dodds, A.W., 1997. The internal thioester and the covalent binding properties of
1058 the complement proteins C3 and C4. Protein Sci 6, 263-274.
1059 Lee, L.Y., Liang, X., Hook, M., Brown, E.L., 2004. Identification and characterization of the C3
1060 binding domain of the Staphylococcus aureus extracellular fibrinogen-binding protein (Efb). J
1061 Biol Chem 279, 50710-50716.
1062 Lehner, P.J., Davies, K.A., Walport, M.J., Cope, A.P., Wurzner, R., Orren, A., Morgan, B.P.,
1063 Cohen, J., 1992. Meningococcal septicaemia in a C6-deficient patient and effects of plasma
1064 transfusion on lipopolysaccharide release. Lancet 340, 1379-1381.
1065 Lewis, L.A., Ram, S., 2014. Meningococcal disease and the complement system. Virulence 5,
1066 98-126.
1067 Lindahl, G., Sjobring, U., Johnsson, E., 2000. Human complement regulators: a major target
1068 for pathogenic microorganisms. Curr Opin Immunol 12, 44-51.
1069 Lintner, K.E., Wu, Y.L., Yang, Y., Spencer, C.H., Hauptmann, G., Hebert, L.A., Atkinson, J.P., Yu,
1070 C.Y., 2016. Early Components of the Complement Classical Activation Pathway in Human
1071 Systemic Autoimmune Diseases. Front Immunol 7, 36.
1072 Liszewski, M.K., Kolev, M., Le Friec, G., Leung, M., Bertram, P.G., Fara, A.F., Subias, M.,
1073 Pickering, M.C., Drouet, C., Meri, S., Arstila, T.P., Pekkarinen, P.T., Ma, M., Cope, A.,
1074 Reinheckel, T., Rodriguez de Cordoba, S., Afzali, B., Atkinson, J.P., Kemper, C., 2013.
1075 Intracellular complement activation sustains T cell homeostasis and mediates effector
1076 differentiation. Immunity 39, 1143-1157.
1077 Liszewski, M.K., Leung, M.K., Hauhart, R., Buller, R.M., Bertram, P., Wang, X., Rosengard,
1078 A.M., Kotwal, G.J., Atkinson, J.P., 2006. Structure and regulatory profile of the monkeypox
1079 inhibitor of complement: comparison to homologs in vaccinia and variola and evidence for
1080 dimer formation. J Immunol 176, 3725-3734.
1081 Lubinski, J., Wang, L., Mastellos, D., Sahu, A., Lambris, J.D., Friedman, H.M., 1999. In vivo role
1082 of complement-interacting domains of herpes simplex virus type 1 glycoprotein gC. J Exp
1083 Med 190, 1637-1646.

Lubinski, J.M., Wang, L., Soulika, A.M., Burger, R., Wetsel, R.A., Colten, H., Cohen, G.H., Eisenberg, R.J., Lambris, J.D., Friedman, H.M., 1998. Herpes simplex virus type 1 glycoprotein gC mediates immune evasion in vivo. J Virol 72, 8257-8263.
Luo, S., Blom, A.M., Rupp, S., Hipler, U.C., Hube, B., Skerka, C., Zipfel, P.F., 2011. The pH-regulated antigen 1 of Candida albicans binds the human complement inhibitor C4b-binding protein and mediates fungal complement evasion. J Biol Chem 286, 8021-8029.
Madico, G., Ngampasutadol, J., Gulati, S., Vogel, U., Rice, P.A., Ram, S., 2007. Factor H binding and function in sialylated pathogenic neisseriae is influenced by gonococcal, but not meningococcal, porin. J Immunol 178, 4489-4497.
Mallery, D.L., McEwan, W.A., Bidgood, S.R., Towers, G.J., Johnson, C.M., James, L.C., 2010. Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). Proc Natl Acad Sci U S A 107, 19985-19990.
Mandrell, R.E., Apicella, M.A., 1993. Lipo-oligosaccharides (LOS) of mucosal pathogens: molecular mimicry and host-modification of LOS. Immunobiology 187, 382-402.
Markiewski, M.M., Lambris, J.D., 2007. The role of complement in inflammatory diseases from behind the scenes into the spotlight. Am J Pathol 171, 715-727.
Marr, N., Shah, N.R., Lee, R., Kim, E.J., Fernandez, R.C., 2011. Bordetella pertussis autotransporter Vag8 binds human C1 esterase inhibitor and confers serum resistance. PLoS One 6, e20585.
Mathew, S., Overturf, G.D., 2006. Complement and properidin deficiencies in meningococcal disease. Pediatr Infect Dis J 25, 255-256.
McEwan, W.A., Tam, J.C., Watkinson, R.E., Bidgood, S.R., Mallery, D.L., James, L.C., 2013. Intracellular antibody-bound pathogens stimulate immune signaling via the Fc receptor TRIM21. Nat Immunol 14, 327-336.
McNamara, L.A., Topaz, N., Wang, X., Hariri, S., Fox, L., MacNeil, J.R., 2017. High Risk for Invasive Meningococcal Disease Among Patients Receiving Eculizumab (Soliris) Despite Receipt of Meningococcal Vaccine. MMWR Morb Mortal Wkly Rep 66, 734-737.
Meri, T., Amdahl, H., Lehtinen, M.J., Hyvarinen, S., McDowell, J.V., Bhattacharjee, A., Meri, S., Marconi, R., Goldman, A., Jokiranta, T.S., 2013. Microbes bind complement inhibitor factor H via a common site. PLoS Pathog 9, e1003308.
Meri, T., Blom, A.M., Hartmann, A., Lenk, D., Meri, S., Zipfel, P.F., 2004. The hyphal and yeast forms of Candida albicans bind the complement regulator C4b-binding protein. Infection and immunity 72, 6633-6641.
Merino, S., Camprubi, S., Alberti, S., Benedi, V.J., Tomas, J.M., 1992. Mechanisms of Klebsiella pneumoniae resistance to complement-mediated killing. Infect Immun 60, 2529-2535.
Merle, N.S., Church, S.E., Fremeaux-Bacchi, V., Roumenina, L.T., 2015. Complement System Part I - Molecular Mechanisms of Activation and Regulation. Front Immunol 6, 262.
Milis, L., Morris, C.A., Sheehan, M.C., Charlesworth, J.A., Pussell, B.A., 1993. Vitronectin-mediated inhibition of complement: evidence for different binding sites for C5b-7 and C9. Clin Exp Immunol 92, 114-119.
Moks, T., Abrahmsen, L., Nilsson, B., Hellman, U., Sjoquist, J., Uhlen, M., 1986. Staphylococcal protein A consists of five IgG-binding domains. Eur J Biochem 156, 637-643.
Mullick, J., Kadam, A., Sahu, A., 2003. Herpes and pox viral complement control proteins: 'the mask of self'. Trends Immunol 24, 500-507.

Nelson, D.C., Garbe, J., Collin, M., 2011. Cysteine proteinase SpeB from Streptococcus pyogenes - a potent modifier of immunologically important host and bacterial proteins. Biol Chem 392, 1077-1088.
Ngampasutadol, J., Ram, S., Gulati, S., Agarwal, S., Li, C., Visintin, A., Monks, B., Madico, G., Rice, P.A., 2008. Human factor H interacts selectively with Neisseria gonorrhoeae and results in species-specific complement evasion. J Immunol 180, 3426-3435.
Nilsson, B., Nilsson Ekdahl, K., 2012. The tick-over theory revisited: is C3 a contact-activated protein? Immunobiology 217, 1106-1110.
Nordstrom, T., Blom, A.M., Tan, T.T., Forsgren, A., Riesbeck, K., 2005. Ionic binding of C3 to the human pathogen Moraxella catarrhalis is a unique mechanism for combating innate immunity. J Immunol 175, 3628-3636.
Noris, M., Remuzzi, G., 2013. Overview of complement activation and regulation. Semin Nephrol 33, 479-492.
O'Hara, A.M., Moran, A.P., Wurzner, R., Orren, A., 2001. Complement-mediated lipopolysaccharide release and outer membrane damage in Escherichia coli J5: requirement for C9. Immunology 102, 365-372.
Olsen, R.J., Raghuram, A., Cantu, C., Hartman, M.H., Jimenez, F.E., Lee, S., Ngo, A., Rice, K.A., Saddington, D., Spillman, H., Valson, C., Flores, A.R., Beres, S.B., Long, S.W., Nasser, W., Musser, J.M., 2015. The majority of 9,729 group A streptococcus strains causing disease secrete SpeB cysteine protease: pathogenesis implications. Infect Immun 83, 4750-4758.
Pangburn, M.K., 2002. Cutting edge: localization of the host recognition functions of complement factor H at the carboxyl-terminal: implications for hemolytic uremic syndrome. J Immunol 169, 4702-4706.
Pausa, M., Pellis, V., Cinco, M., Giulianini, P.G., Presani, G., Perticarari, S., Murgia, R., Tedesco, F., 2003. Serum-resistant strains of Borrelia burgdorferi evade complement-mediated killing by expressing a CD59-like complement inhibitory molecule. J Immunol 170, 3214-3222.
Perez, J.L., Absalon, J., Beeslaar, J., Balmer, P., Jansen, K.U., Jones, T.R., Harris, S., York, L.J., Jiang, Q., Radley, D., Anderson, A.S., Crowther, G., Eiden, J.J., 2018. From research to licensure and beyond: clinical development of MenB-FHbp, a broadly protective meningococcal B vaccine. Expert Rev Vaccines 17, 461-477.
Persson, J., Beall, B., Linse, S., Lindahl, G., 2006. Extreme sequence divergence but conserved ligand-binding specificity in Streptococcus pyogenes M protein. PLoS Pathog 2, e47.
Potempa, J., Pike, R.N., 2009. Corruption of innate immunity by bacterial proteases. J Innate Immun 1, 70-87.
Preissner, K.T., 1991. Structure and biological role of vitronectin. Annu Rev Cell Biol 7, 275-310.
Preissner, K.T., Seiffert, D., 1998. Role of vitronectin and its receptors in haemostasis and vascular remodeling. Thromb Res 89, 1-21.
Ram, S., Gulati, S., Lewis, L.A., Chakraborti, S., Zheng, B., DeOliveira, R.B., Reed, G.W., Cox, A.D., Li, J., St Michael, F., Stupak, J., Su, X.H., Saha, S., Landig, C.S., Varki, A., Rice, P.A., 2018. A Novel Sialylation Site on Neisseria gonorrhoeae Lipooligosaccharide Links Heptose II Lactose Expression with Pathogenicity. Infect Immun 86.
Ram, S., Lewis, L.A., Rice, P.A., 2010. Infections of people with complement deficiencies and patients who have undergone splenectomy. Clin Microbiol Rev 23, 740-780.

Ram, S., Sharma, A.K., Simpson, S.D., Gulati, S., McQuillen, D.P., Pangburn, M.K., Rice, P.A., 1998. A novel sialic acid binding site on factor H mediates serum resistance of sialylated Neisseria gonorrhoeae. J Exp Med 187, 743-752.
Ram, S., Shaughnessy, J., DeOliveira, R.B., Lewis, L.A., Gulati, S., Rice, P.A., 2016. Utilizing complement evasion strategies to design complement-based antibacterial immunotherapeutics: Lessons from the pathogenic Neisseriae. Immunobiology 221, 1110-1123.
Ramu, P., Tanskanen, R., Holmberg, M., Lahteenmaki, K., Korhonen, T.K., Meri, S., 2007. The surface protease PgtE of Salmonella enterica affects complement activity by proteolytically cleaving C3b, C4b and C5. FEBS Lett 581, 1716-1720.
Rappuoli, R., Pizza, M., Masignani, V., Vadivelu, K., 2018. Meningococcal B vaccine (4CMenB): the journey from research to real world experience. Expert Rev Vaccines 17, 1111-1121.
Ricklin, D., Hajishengallis, G., Yang, K., Lambris, J.D., 2010. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 11, 785-797.
Ricklin, D., Lambris, J.D., 2016. New milestones ahead in complement-targeted therapy. Semin Immunol 28, 208-222.
Ricklin, D., Mastellos, D.C., Reis, E.S., Lambris, J.D., 2018. The renaissance of complement therapeutics. Nat Rev Nephrol 14, 26-47.
Roberts, I.S., 1996. The biochemistry and genetics of capsular polysaccharide production in bacteria. Annu Rev Microbiol 50, 285-315.
Roberts, I.S., Saunders, F.K., Boulnois, G.J., 1989. Bacterial capsules and interactions with complement and phagocytes. Biochem Soc Trans 17, 462-464.
Rooijakkers, S.H., Ruyken, M., Roos, A., Daha, M.R., Presanis, J.S., Sim, R.B., van Wamel, W.J., van Kessel, K.P., van Strijp, J.A., 2005. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat Immunol 6, 920-927.
Rooijakkers, S.H., Wu, J., Ruyken, M., van Domselaar, R., Planken, K.L., Tzekou, A., Ricklin, D., Lambris, J.D., Janssen, B.J., van Strijp, J.A., Gros, P., 2009. Structural and functional implications of the alternative complement pathway C3 convertase stabilized by a staphylococcal inhibitor. Nat Immunol 10, 721-727.
Sahly, H., Keisari, Y., Ofek, I., 2009. Manno(rhamno)biose-containing capsular polysaccharides of Klebsiella pneumoniae enhance opsono-stimulation of human polymorphonuclear leukocytes. J Innate Immun 1, 136-144.
Sahu, A., Kozel, T.R., Pangburn, M.K., 1994. Specificity of the thioester-containing reactive site of human C3 and its significance to complement activation. Biochem J 302 (Pt 2), 429-436.
Schmidt, C.Q., Kennedy, A.T., Tham, W.H., 2015. More than just immune evasion: Hijacking complement by Plasmodium falciparum. Mol Immunol 67, 71-84.
Schneider, M.C., Prosser, B.E., Caesar, J.J., Kugelberg, E., Li, S., Zhang, Q., Quoraishi, S., Lovett, J.E., Deane, J.E., Sim, R.B., Roversi, P., Johnson, S., Tang, C.M., Lea, S.M., 2009. Neisseria meningitidis recruits factor H using protein mimicry of host carbohydrates. Nature 458, 890-893.
Schorey, J.S., Carroll, M.C., Brown, E.J., 1997. A macrophage invasion mechanism of pathogenic mycobacteria. Science 277, 1091-1093.
Seth, R.B., Sun, L., Ea, C.K., Chen, Z.J., 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122, 669-682.

Shayakhmetov, D.M., Gaggar, A., Ni, S., Li, Z.Y., Lieber, A., 2005. Adenovirus binding to blood factors results in liver cell infection and hepatotoxicity. J Virol 79, 7478-7491.
Singh, B., Su, Y.C., Riesbeck, K., 2010. Vitronectin in bacterial pathogenesis: a host protein used in complement escape and cellular invasion. Mol Microbiol 78, 545-560.
Sjodahl, J., 1977. Repetitive sequences in protein A from Staphylococcus aureus. Arrangement of five regions within the protein, four being highly homologous and Fc-binding. Eur J Biochem 73, 343-351.
Slade, C., Bosco, J., Unglik, G., Bleasel, K., Nagel, M., Winship, I., 2013. Deficiency in complement factor B. N Engl J Med 369, 1667-1669.
Smith, H., Parsons, N.J., Cole, J.A., 1995. Sialylation of neisserial lipopolysaccharide: a major influence on pathogenicity. Microb Pathog 19, 365-377.
Sorbara, M.T., Foerster, E.G., Tsalikis, J., Abdel-Nour, M., Mangiapane, J., Sirluck-Schroeder, I., Tattoli, I., van Dalen, R., Isenman, D.E., Rohde, J.R., Girardin, S.E., Philpott, D.J., 2018. Complement C3 Drives Autophagy-Dependent Restriction of Cyto-invasive Bacteria. Cell Host Microbe 23, 644-652 e645.
Spitzer, D., Mitchell, L.M., Atkinson, J.P., Hourcade, D.E., 2007. Properdin can initiate complement activation by binding specific target surfaces and providing a platform for de novo convertase assembly. J Immunol 179, 2600-2608.
Sprong, T., Roos, D., Weemaes, C., Neeleman, C., Geesing, C.L., Mollnes, T.E., van Deuren, M., 2006. Deficient alternative complement pathway activation due to factor D deficiency by 2 novel mutations in the complement factor D gene in a family with meningococcal infections. Blood 107, 4865-4870.
Steimle, A., Autenrieth, I.B., Frick, J.S., 2016. Structure and function: Lipid A modifications in commensals and pathogens. Int J Med Microbiol 306, 290-301.
Tam, J.C., Bidgood, S.R., McEwan, W.A., James, L.C., 2014. Intracellular sensing of complement C3 activates cell autonomous immunity. Science 345, 1256070.
Tan, L.A., Yang, A.C., Kishore, U., Sim, R.B., 2011. Interactions of complement proteins C1q and factor H with lipid A and Escherichia coli: further evidence that factor H regulates the classical complement pathway. Protein Cell 2, 320-332.
Terao, Y., Mori, Y., Yamaguchi, M., Shimizu, Y., Ooe, K., Hamada, S., Kawabata, S., 2008. Group A streptococcal cysteine protease degrades C3 (C3b) and contributes to evasion of innate immunity. J Biol Chem 283, 6253-6260.
Terao, Y., Yamaguchi, M., Hamada, S., Kawabata, S., 2006. Multifunctional glyceraldehyde-3-phosphate dehydrogenase of Streptococcus pyogenes is essential for evasion from neutrophils. J Biol Chem 281, 14215-14223.
Tesh, V.L., Duncan, R.L., Jr., Morrison, D.C., 1986. The interaction of Escherichia coli with normal human serum: the kinetics of serum-mediated lipopolysaccharide release and its dissociation from bacterial killing. J Immunol 137, 1329-1335.
Tham, W.H., Schmidt, C.Q., Hauhart, R.E., Guariento, M., Tetteh-Quarcoo, P.B., Lopaticki, S., Atkinson, J.P., Barlow, P.N., Cowman, A.F., 2011. Plasmodium falciparum uses a key functional site in complement receptor type-1 for invasion of human erythrocytes. Blood 118, 1923-1933.
Tham, W.H., Wilson, D.W., Lopaticki, S., Schmidt, C.Q., Tetteh-Quarcoo, P.B., Barlow, P.N., Richard, D., Corbin, J.E., Beeson, J.G., Cowman, A.F., 2010. Complement receptor 1 is the host erythrocyte receptor for Plasmodium falciparum PfRh4 invasion ligand. Proc Natl Acad Sci U S A 107, 17327-17332.

Thammavongsa, V., Kim, H.K., Missiakas, D., Schneewind, O., 2015. Staphylococcal manipulation of host immune responses. Nat Rev Microbiol 13, 529-543.
Tsao, N., Tsai, W.H., Lin, Y.S., Chuang, W.J., Wang, C.H., Kuo, C.F., 2006. Streptococcal pyrogenic exotoxin B cleaves properdin and inhibits complement-mediated opsonophagocytosis. Biochem Biophys Res Commun 339, 779-784.
Tschopp, J., Chonn, A., Hertig, S., French, L.E., 1993. Clusterin, the human apolipoprotein and complement inhibitor, binds to complement C7, C8 beta, and the b domain of C9. J Immunol 151, 2159-2165.
Vogl, G., Lesiak, I., Jensen, D.B., Perkhofer, S., Eck, R., Speth, C., Lass-Florl, C., Zipfel, P.F., Blom, A.M., Dierich, M.P., Wurzner, R., 2008. Immune evasion by acquisition of complement inhibitors: the mould Aspergillus binds both factor H and C4b binding protein. Mol Immunol 45, 1485-1493.
Weismann, D., Hartvigsen, K., Lauer, N., Bennett, K.L., Scholl, H.P., Charbel Issa, P., Cano, M., Brandstatter, H., Tsimikas, S., Skerka, C., Superti-Furga, G., Handa, J.T., Zipfel, P.F., Witztum, J.L., Binder, C.J., 2011. Complement factor H binds malondialdehyde epitopes and protects from oxidative stress. Nature 478, 76-81.
Wickham, S.E., Hotze, E.M., Farrand, A.J., Polekhina, G., Nero, T.L., Tomlinson, S., Parker, M.W., Tweten, R.K., 2011. Mapping the intermedilysin-human CD59 receptor interface reveals a deep correspondence with the binding site on CD59 for complement binding proteins C8alpha and C9. J Biol Chem 286, 20952-20962.
Woehl, J.L., Ramyar, K.X., Katz, B.B., Walker, J.K., Geisbrecht, B.V., 2017. The structural basis for inhibition of the classical and lectin complement pathways by S. aureus extracellular adherence protein. Protein Sci 26, 1595-1608.
Woehl, J.L., Stapels, D.A.C., Garcia, B.L., Ramyar, K.X., Keightley, A., Ruyken, M., Syriga, M., Sfyroera, G., Weber, A.B., Zolkiewski, M., Ricklin, D., Lambris, J.D., Rooijakkers, S.H.M., Geisbrecht, B.V., 2014. The extracellular adherence protein from Staphylococcus aureus inhibits the classical and lectin pathways of complement by blocking formation of the C3 proconvertase. J Immunol 193, 6161-6171.
Yang, Y., Back, C.R., Grawert, M.A., Wahid, A.A., Denton, H., Kildani, R., Paulin, J., Worner, K., Kaiser, W., Svergun, D.I., Sartbaeva, A., Watts, A.G., Marchbank, K.J., van den Elsen, J.M.H., 2018. Utilization of Staphylococcal Immune Evasion Protein Sbi as a Novel Vaccine Adjuvant. Front Immunol 9, 3139.
Zipfel, P.F., Skerka, C., 2009. Complement regulators and inhibitory proteins. Nat Rev Immunol 9, 729-740.

Figure Legends

Figure 1: Activated complement cascade on a surface. Schematic representation of the complement cascade. Complement can be activated by three independent pathways: Classical pathway (CP) through IgG's; Lectin Pathway (LP) via carbohydrates (both on the left side) or Alternative Pathway (AP) through spontaneous tick-over and probing of surfaces (upper part in the middle). All pathways converge at the level of C3 convertases leading to the opsonisation of the target (middle of scheme) and progressing via C5 convertases to the terminal pathway which results in the generation of the membrane attack complex (MAC).

Complement inhibition by host molecules (soluble and surface bound) is highlighted by red lines.

Figure 2: Factor H: structure and binding sites for virulence factors of human pathogens. Schematic representation of the soluble complement inhibitor of the AP, Factor H (FH). FH is composed of 20 CCP domains. FH binds to host cell surfaces, specifically to glycosaminoglycans (GAG) via domains 6-7 and 19-20. C3b binding is mediated through domains 19-20 and 1-4. CCP1-4 also mediates the complement regulatory function (domains highlighted in green). Pathogens bind to all CCP domains of FH, with a strong affinity for domains 5-7 and 19-20. The bars behind the pathogens name indicate the different CCP domains which are targeted by that pathogen.

Figure 3: C4b-binding protein: structure and binding sites for virulence factors of human pathogens. (**A**) Schematic representation of the soluble complement inhibitor of the CP and LP, C4b-binding protein (C4BP). C4BP is composed of 7 α-chains and one β-chain ($\alpha7\beta1$), but can also be found in a $\alpha7\beta0$ configuration, lacking the β-chain. (**B**) Each α-chain consists of 8 CCP domains. CCP1-3 mediate the complement regulatory function (domains highlighted in green). Beside binding C4b (CCP1-3) and C3b (CCP1-4), pathogens do also bind to different CCP domains, indicated by the bars after the pathogens name.

Figure 4: Plasminogen activation on pathogens protects from complement activation. Plasminogen (PLG) can be bound and directly activated by different surface virulence factors and (e.g. omptins; left side). Omptins can also enhance plasmin activity by degrading $\alpha2$-antiplasmin, a host plasmin inhibitor. Other bacterial plasminogen receptors only bind plasminogen, which then becomes activated by host serum factors, such as tPA or uPA (right side). Both cases result in a cleavage of C4b, C3b and C5, which inactivates complement and prevents opsonisation and anaphylatoxin release.

Figure 5: Intracellular complement and clearance of pathogens. Before invading a cell, bacteria are exposed to complement and eventually opsonised (**A**). As soon as those bacteria invade cells and reach the cytoplasm, ATG16L1 recognizes C3b and induces autophagy, which leads to the destruction of the pathogen (exemplary shown for Listeria as blue bacteria). However, if C3b is degraded, ATG16L1 does not recognize C3b and bacteria evade autophagy thus being able to replicate (shown here for Shigella in orange). Similarly, non-enveloped viruses are recognized and can be opsonised before entering the cell (**B**). Once in the cytosol, MAVS recognize C3b deposition on the virus and induce translation of genes such as IRF, NFκb and AP-1.

Figure 6: Summary of how pathogens evade complement. (**A**) Increased complement activation always leads to a decreased microbial survival due to selective pressure. For pathogens to survive, efficient complement evasive strategies are necessary. (**B**) Pathogens release soluble virulence factors, such as inhibitors, proteases or other factors that directly degrade complement or activate it in a place remote from the pathogen (left side of scheme). Inhibition of complement can also be caused by recruiting different host serum factors which interfere with complement activation due to protease activity or regulatory domains (right side of scheme). In both cases, complement is inhibited on different stages, since nearly all complement proteins can be targeted. This prevents complement recognition, opsonisation, immune activation and MAC deposition (top part of scheme).

1360