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REVIEW ARTICLE

Complement interactions with the pathogenic Neisseriae: clinical features, deficiency states, and evasion mechanisms

Lisa A. Lewis and Sanjay Ram

Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA, USA

Correspondence
L. A. Lewis and S. Ram, Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01605, USA
Tel: +1 508 856 6269
E-mails: Lisa.lewis@umassmed.edu (LAL); Sanjay.ram@umassmed.edu (SR)

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Neisseria gonorrhoeae **causes the sexually transmitted infection gonorrhea, while** ***Neisseria meningitidis*** **is an important cause of bacterial meningitis and sepsis. Complement is a central arm of innate immune defenses and plays an important role in combating Neisserial infections. Persons with congenital and acquired defects in complement are at a significantly higher risk for invasive Neisserial infections such as invasive meningococcal disease and disseminated gonococcal infection compared to the general population. Of note,** ***Neisseria gonorrhoeae*** **and** ***Neisseria meningitidis*** **can only infect humans, which in part may be related to their ability to evade only human complement. This review summarizes the epidemiologic and clinical aspects of Neisserial infections in persons with defects in the complement system. Mechanisms used by these pathogens to subvert killing by complement and preclinical studies showing how these complement evasion strategies may be used to counteract the global threat of meningococcal and gonococcal infections are discussed.**

Keywords: alternative pathway; antibody; C4b-binding protein; classical pathway; complement; factor H; lectin pathway; *Neisseria gonorrhoeae*; *Neisseria meningitidis*

The complement system evolved from a rudimentary system comprised of only a few key initiation molecules of the alternative pathway (AP; see Box 1 and Fig. 1 for a summary of the complement system) that date as far back as sponges (> 400 million years ago; Porifera) [1] to a complex system of over 60 soluble and membrane-associated proteins that act in a coordinated manner to combat invading microbes. Work over the past couple of decades has shown that the functions of complement extend well beyond pathogen surveillance to include bridging innate and acquired immune systems, disposal of immune complexes and apoptotic cells, metabolism, and tissue regeneration and organogenesis [2,3].

It is no surprise that microbes that have coevolved with their hosts have developed elegant and often redundant mechanisms to avoid detection and killing by host complement [4]. In many instances, microbes

Abbreviations
CP, classical pathway; AP, alternative pathway; LP, lectin pathway; MBL, mannose-binding lectin; MASP, MBL-associated serine protease; FB, factor B; FD, factor D; FH, factor H; FI, factor I; MAC, membrane attack complex; CR3, complement receptor 3; C5aR1, C5a receptor 1; CCP, complement control protein; PNH, paroxysmal nocturnal hemoglobinuria; aHUS, atypical uremic syndrome; DGI, disseminated gonococcal infection; FHbp, factor H binding protein; NspA, Neisserial surface protein A; NHBA, Neisserial heparin binding antigen; mAb, monoclonal antibody; LOS, lipooligosaccharide; Hep, heptose; Glc, glucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; LNnT, lacto-*N*-neotetraose; Neu5Ac, *N*-acetylneuraminic acid; CMP-Neu5Ac, cytidine monophospho-*N*-acetylneuraminic acid; Por, Porin; Opa, Opacity protein; Rmp, reduction-modifiable protein; Lgt, lipooligosaccharide glycosyl transferase; PEtn, phosphoethanolamine.

Box 1. Activation of the complement cascade1

- Complement activation is initiated through three pathways—the classical (CP), lectin (LP), or alternative pathways (AP) (Fig. 1).
- The CP is initiated when antibodies (IgM or IgG) bind to pathogens, which then engages C1q in the C1 complex. C1r and C1s, serine proteases in the C1 complex, are activated by conformational changes when C1q binds to immunoglobulins. C1s cleaves C4; the C4b fragment binds covalently to the microbial surface. C2 then binds to C4b, which leads to formation of the CP C3 convertase, C4bC2a.
- The LP is activated by binding of mannose-binding lectin (MBL), ficolins or collectins and their accompanying MBL-associated serine proteases (MASPs) to surfaces to activate C4 or C3, similar to CP activation.
- AP activation occurs when factor B (FB) binds to C3b (generated by 'tickover'; spontaneous hydrolysis of C3, or *via* CP/LP activation); C3bB is cleaved by the enzyme factor D (FD) to form the AP C3 convertase, C3bBb. Properdin stabilizes the AP C3 convertase and extends its inherently short half-life (~ 90 s) by 5- to 10-fold. AP C3 convertase cleaves more C3 to initiate a positive feedback loop that amplifies C3 deposition.
- C3 is the 'central hub' of complement activation. C3 fragments mark microbes for uptake by phagocytes through complement receptors (CRs) such as CR3.
- The addition of a C3b molecule to CP and AP C3 convertases imparts the ability to cleave C5 (C5 convertase activity); the C5a fragment is a potent anaphylatoxin and chemotaxin that facilitates opsonophagocytosis, while C5b recruits C6 through C9 to form C5b-9, the membrane attack complex (MAC) pore, which can kill of gram-negative bacteria.
- Excessive complement activation is prevented by several soluble (e.g., C1 inhibitor, C4 binding protein [C4BP], Factor H [FH], and Factor I; Fig. 1, green) and membrane-associated complement inhibitors.

employ similar strategies as their hosts to limit complement activation on their surfaces [5,6]. This review will discuss the importance of complement in host defenses against Neisserial infections, as evidenced by the association between congenital and acquired defects of complement and incidence of invasive Neisserial disease, and will also discuss strategies that meningococci and gonococci use to evade the human complement system.

Complement deficiencies and Neisserial infections

Congenital deficiencies of complement and Neisserial infections

Inherited deficiencies of complement are uncommon, with an overall prevalence in the general population of about 0.03% [7]. The incidence of deficiencies of individual complement components varies according to the ethnicity of the population studied. Complete deficiency of C2 is the most common inherited deficiency of complement in Caucasians, with an incidence of approximately 0.01%. C9 deficiency, which is extremely rare in Caucasians, was detected in about 0.1% of Japanese blood donors [7]. Most cases of complement deficiency are diagnosed because of their association with autoimmune diseases and/or recurrent bacterial infections.

Deficiencies of classical pathway (CP) are associated with autoimmune disorders [7,8]. The incidence of autoimmune disease varies with the deficient component and, in general, decreases with advancement down the cascade: almost every human with C1q deficiency, over 75% of all individuals with total C4 deficiency, 57% of individuals with C1r/s deficiency, and only about 10% of persons with homozygous C2 deficiency have autoimmune disorders [9–11].

Classical pathway deficiencies are also associated with respiratory infections and sepsis caused by encapsulated pathogens, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *N. meningitidis* [7,12]. Three cases of invasive meningococcal infection were reported among 40 individuals with homozygous C2 deficiency from 33 Swedish families over a 25-year period [13]. A follow-up study of the Swedish C2-deficient cohort showed that homozygosity of G2m(n), an allotype marker of the IgG2 heavy chain, was associated with less severe infections [14]. Possible reasons for the negative association between infections and the G2m (n) allotype include the higher antibody responses to immunization with polysaccharides in these individuals [15–17] and direct activation of the alternative pathway (i.e., by 'bypassing' the classical pathway) by anticapsular antibodies [18,19].

Case reports have linked mannose-binding lectin (MBL) deficiency with meningococcal infection

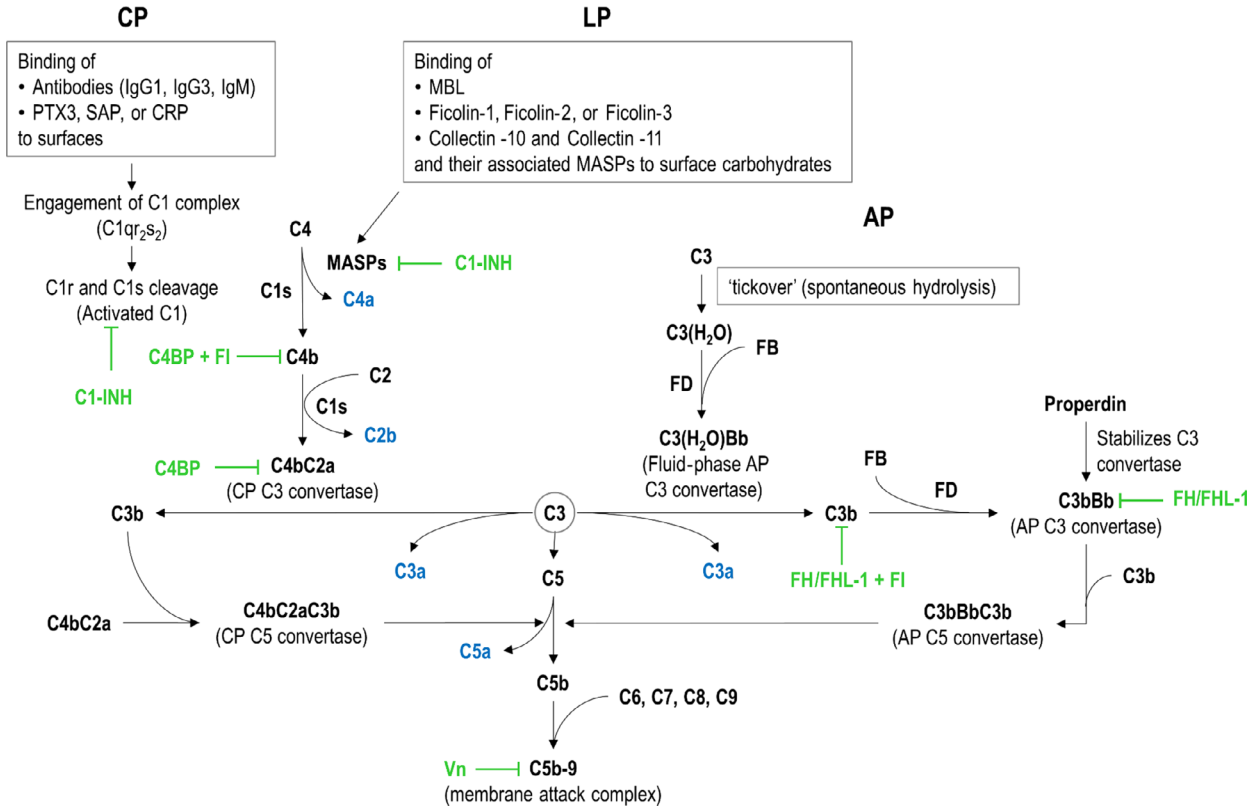


Fig. 1. Schematic representing the activation of the complement cascade. The fragments released into solution are indicated in blue font. The key fluid-phase regulators are indicated in green font. AP, alternative pathway; C1-INH, C1 inhibitor; C4BP, C4b-binding protein; CP, classical pathway; CRP, C-reactive protein; FB, factor B; FD, factor D; FH, factor H; FHL-1, factor H like protein-1; FI, factor I; LP, lectin pathway; MASP, MBL-associated serine protease; MBL, mannan-binding lectin; PTX3, pentraxin 3; SAP, serum amyloid P component; Vn, vitronectin.

[20,21]. The frequency of genetic variants of the *MBL* gene that predicted low plasma MBL levels was higher in persons with meningococcal disease than in control patients (odds ratio 2.4) [22]. MBL deficiency was associated with higher bacterial DNA loads measured in plasma during meningococcal infection, and the effect was greatest in children under the age of 2 years [23]. However, this study did not report an association between MBL genotypes that predict MBL deficiency and an increased risk of infection, even in infants. Cohorts in Norway [24] and a large study in Denmark also did not associate *MBL* genotypes predictive of MBL deficiency with meningococcal infection [25]. Thus, the role of MBL in host defenses against meningococcal infection remains controversial.

Alternative pathway (AP) deficiencies are strongly associated with meningococcal infections. Deficiencies in activators of the AP such as factor D (FD), the enzyme that cleaves factor B (FB) or properdin, the most common inherited alternative pathway deficiency and the only X-linked complement deficiency, are associated with invasive meningococcal disease [7,26–33]. FB deficiency is rare; however, the only described individual with FB deficiency suffered two episodes each of invasive pneumococcal and meningococcal disease [34].

Defects of terminal complement components (C5 through C9) are almost exclusively associated with increased risk from recurrent invasive meningococcal disease and disseminated gonococcal infection [7,12,31]. Terminal complement component-deficient individuals have a 5000- to 10 000-fold greater risk of contracting meningococcal diseases than normal persons. In addition, uncommon serogroups and nongroupable (unencapsulated) isolates may be encountered more frequently in terminal pathway-deficient patients than in normal individuals [35,36]. The median age of presentation of meningococcal infection in terminal pathway deficiency is 17 years, compared to 3 years in normal individuals [7,31]. Recurrence of diseases is another characteristic feature;

almost 50% of individuals with deficiencies in components of the terminal pathway will experience at least one additional episode. C9-deficient persons may be less predisposed to invasive meningococcal infections and also less prone to recurrent infections compared to persons with deficiencies of C5 through C8; only 9% (15 of 165) C9-deficient patients suffered meningococcal infections and no cases of recurrences were reported, compared to a disease incidence of 58% (146 of 250) in C5- to C8-deficient persons, and a recurrence rate of 45% [31]. While C8-deficient serum did not support bactericidal activity, C9-deficient serum (or normal serum depleted of C9) had delayed, but present, bactericidal activity against *N. gonorrhoeae* and *N. meningitidis in vitro* [37]. Thus, C9 was not required for killing of Neisseriae, which could account for lower predisposition of meningococcal disease in C9-deficient individuals.

Curiously, persons with inherited terminal component deficiency experience a lower mortality per meningococcal episode compared to complement-sufficient patients—a 1.5% mortality compared to 19% mortality per 100 episodes [7,31,38,39]. Mortality and severity of meningococcal disease is proportional to complement activation and endotoxin levels in plasma [40]. Lower mortality in terminal complement deficiency may be linked to lower endotoxin levels in these patients [41]. Experiments *in vitro* with *E. coli* J5 have shown that an intact complement system is associated with the release of LPS from the bacterial membrane and inability to form the MAC pore markedly decreases LPS release [42,43].

Acquired deficiencies of complement and Neisserial infections

Acquired defects of complement may result from decreased synthesis (e.g., severe liver disease), increased consumption (e.g., vasculitis and immune complex disease), or increased catabolism (e.g., nephrotic syndrome and protein-losing enteropathies). Cases of meningococcemia have been reported at an increased incidence in persons with acquired defects of complement, such as end-stage liver disease, multiple myeloma, and lupus [44–46] when compared to incidence in normal individuals. Ellison and colleagues found that 6 of 20 individuals with their first episode of meningococcal disease had CH_{50} (the dilution of serum that lyses 50% of RBCs in the reaction mixture) levels ≥ 2 standard deviations below the normal mean [45]. Three patients had defects of the terminal pathway, two systemic lupus erythematosus and one had multiple myeloma [45].

Excessive activation of complement contributes to the pathogenesis of several diseases, including paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), and cold agglutinin disease. Several inhibitors of complement are currently in clinical trials to mitigate these conditions. Eculizumab, which blocks the cleavage of C5, was first approved for clinical use in 2007 and, to date, remains the only FDA-approved complement inhibitor. Seventy-six cases of meningococcal disease were reported following 28 518 patient-years of drug exposure—a disease incidence of 0.25/100 patient-years, which is about 2000-fold higher than that of the general population [47]. Cases of invasive meningococcal disease occurred despite the administration of the recommended meningococcal vaccines [48,49]. Individuals on eculizumab treatment mounted adequate antibody responses to vaccines, which was expected to confer protection through opsonophagocytosis. However, eculizumab blocks cleavage of C5 and the ensuing absence of C5a—which is critical for the activation of neutrophils and the oxidative burst [50]—greatly decreases killing of meningococci by whole blood *in vitro* [51]. By contrast, blocking C7 (and thereby maintaining the ability to generate C5a) preserved opsonophagocytic killing by whole blood [51]. Eculizumab treatment is also associated with a high risk of disseminated gonococcal infection (DGI) [52], as well as bacteremia caused by commensal Neisserial species [53]—for reasons not clear, some of the DGI cases were unexpectedly severe [54].

Complement activation on *Neisseria*

The classical pathway (CP)

With the exception of some instances of anticapsular antibody-induced AP activation that can kill meningococci [18,19], an intact CP is necessary to kill Neisseriae, evidenced by the lack of bactericidal activity *in vitro* with use of IgG- and IgM-depleted serum [55,56], serum absorbed against the homologous strain to deplete strain-specific antibody [57,58] or agammaglobulinenic serum [57], Mg-EGTA-treated serum (chelates Ca^{2+} and blocks the classical and lectin pathways) [59], and C1q depletion [60].

The ability of an antibody to activate the CP depends on several factors, including the density and distribution of the target antigen, accessibility of the target antigen, avidity of antibody binding, and antibody subclass. Elegant structural studies showed that IgG forms ordered hexamers through noncovalent interactions between Fc domains, which results in

engagement of the 6 globular heads of C1q in the C1 complex by each of the Fc's in the hexamer [61]. Engagement of the C1 complex by Fc is followed by cleavage of C4, which results in the C4b fragment deposited covalently (either *via* an ester or amide bond as discussed below) to a proximate bacterial target. C4b then recruits C2, and the latter is also cleaved by C1s. Therefore, high-avidity antibodies are not likely to be easily displaced from the surface and will activate complement more effectively. A comparison of the bactericidal efficacy of murine mAbs (IgG2a) and a human IgG3λ that recognized overlapping epitopes on porin A (PorA) showed that only the murine mAbs were functional; while the human and murine mAbs showed similar affinity, the murine mAbs possessed higher avidity [62]. Studies comparing chimeric mAbs directed against PorA or meningococcal factor H binding protein (FHbp), where each set of mAbs possessed the same variable regions has revealed that differences in subclass and the hinge region modulate bactericidal and opsonophagocytic function [63–66]. A chimeric mAb with human IgG1 Fc directed against meningococcal PorA, an abundant membrane antigen, showed similar or greater bactericidal activity than the corresponding chimeric mAb with human IgG3. However, a chimeric mAb directed against meningococcal FHbp that is expressed less abundantly than PorA showed greater activity when its Fc was human IgG3 compared to the corresponding IgG1 chimeric [63,64]. IgG3 has a longer hinge region (62 amino acids) than IgG1 (15 amino acids). Interestingly, truncating the hinge region of IgG3 to 15 or 17 amino acids enhanced the bactericidal activity of the antimeningococcal chimeric mAbs, regardless of their targets [63]. Further, the ability of anti-FHbp mAbs to block factor H (FH, AP inhibitor) binding to the bacterial surface reverses AP inhibition and contributes to enhanced bactericidal efficacy of these mAbs [67,68]. The contribution of the AP in amplifying the CP is discussed below.

Recent work in the mouse vaginal colonization model has shed light on the role of antibody-mediated CP activation in protection against experimental gonorrhea [56]. A human IgG1 chimeric mAb directed against gonococcal lipooligosaccharide (LOS) was effective in attenuating colonization of mouse vaginas only when the mAb possessed bactericidal activity. Further, mutations in the Fc region that increased the ability of the mAb to form hexamers enhanced activity of the mAb both *in vitro* and *in vivo*. Activity of the mAb in mice required an intact classical pathway, as well as an intact terminal pathway, evidenced by complete loss of activity in $C1q^{-/-}$ and $C9^{-/-}$ mice, and mice where C5 cleavage was blocked [56]. However, activity of the mAb was unaffected in mice depleted of neutrophils or when C5a receptor 1 (C5aR1) was blocked. Of note, the mAb was also effective when administered systemically (intravenously), consistent with the abundance of the neonatal Fc receptor (FcRn) in genital epithelial cells that mediates bidirectional transfer of IgG across the epithelial barrier [69], resulting in IgG as the dominant antibody subclass in human genital secretions [70,71]. Hemolytically active complement is present in female human cervical secretions—the lytic activity of midcycle cervical mucus was 11.5% of that present in an equal volume of undiluted human serum [72]. All components of the AP are secreted by primary human vaginal and cervical epithelial cells [73]. Several other cell types also secrete various complement components [74,75]. Gonococci recovered directly from human and mouse secretions are coated with antibody and complement components C3 and C4 [76,77]. Collectively, these data provide evidence that bactericidal antibodies in conjunction with complement may be protective against gonorrhea in the female genital tract.

The lectin pathway (LP)

Purified MBL binds to various strains of *N. meningitidis*; group A strains were noted to be high binders [78], while expression of group B meningococcal capsular polysaccharide inhibited the binding of MBL to bacteria in one study [78]. A separate study by Jack and colleagues showed that binding of MBL was not inhibited by group B capsule, but was inhibited by LOS sialylation [79]. Porin B (PorB) and opacity protein (Opa) have also been identified as ligands for MBL on *N. meningitidis* [80]. MBL bound to an MBL serine protease (MBL-MASP complexes) activated C4 and resulted in deposition of C4b on bacteria. Despite its ability to bind to the bacterial surface, opsonization of group A meningococci and unencapsulated meningococcal mutants with MBL did not enhance opsonophagocytosis with human neutrophils [81].

Purified MBL was also shown to bind to *N. gonorrhoeae* and, as with *N. meningitidis*, sialylation of the lacto-*N*-neotetraose (LNnT) extension from heptose I (HepI) of LOS decreased MBL binding [82]. Gonococcal mutants engineered so that LOS HepI glycan terminated in GlcNAc bound high amounts of MBL [83]. Killing of gonococci occurred only when bacteria were pre-incubated with MBL-MASP, followed by addition of MBL-deficient serum, but not when MBL-deficient serum was first reconstituted with MBL-MASP and then added to bacteria. C1 inhibitor (C1 INH) and α_2-

macroglobulin inhibited MBL-MASP activity on the gonococcal surface [83]. Collectively, the evidence thus far suggests that the MBL pathway alone may not support direct complement-dependent killing or opsonophagocytosis of Neisseriae in the context of serum.

The alternative pathway (AP)

The AP alone (in the absence of IgG and IgM) mediates negligible C3 deposition on Neisseriae and cannot support complement-dependent killing; however, in the presence of Ab, it is important for amplification of C3b deposited through the classical pathway. The bactericidal efficacy of antibodies directed against abundantly expressed meningococcal surface antigens such as PorA is unaffected when the AP is blocked [64]. However, antibodies directed against sparsely expressed meningococcal surface antigens such as FHbp require AP recruitment (including the participation of properdin) for maximal bactericidal activity [64]. Overexpression of FHbp abrogated the need for AP recruitment for maximal killing activity, demonstrating that kinetically overwhelming CP activation can compensate for the lack of a functional AP [64].

Antibodies can directly activate the AP in the absence of C1, C4, or C2 [84–90]. Activation of the AP requires $F(ab')_2$, but not Fc, thereby ruling out participation of the C1 complex. Antibodies against capsular polysaccharides of Type III group B streptococci [91], *Haemophilus influenzae* type b [92], *Streptococcus pneumoniae* [19,93], and *N. meningitidis* [18] all can activate the AP. The C_H1 region of IgG contains a preferred binding site for C3b [94,95]. C3b bound to IgG is relatively resistant to inactivation by factors H and I and can associate with factor B and properdin to form a C3 convertase and amplify complement activation [96,97]. This mechanism of complement activation may confer protection against meningococcal infection in immunized individuals even in the absence of a functional CP. Understanding the role of Ab and the AP in killing *Neisseria* will be increasingly important as pathway-specific complement inhibitors enter the clinical market.

Properdin is a key positive regulator of the AP that increases the half-life of both C3 and C5 convertases and an AP initiator. Spitzer and colleagues showed that commercially available purified properdin bound to *N. gonorrhoeae* and activated the AP [98]. Here, surface-associated properdin bound C3b and served as a platform for assembly of AP C3 convertase [98]. However, commercial sources of properdin contain aggregates or high-order oligomers of properdin, in addition to the physiological 'native' (dimeric, trimeric, and tetrameric) forms [99,100]. These nonphysiologic aggregates may have higher avidity and perhaps exhibit nonspecific binding to Neisseriae. Native properdin does not bind directly to gonococci or meningococci, but remains critical for optimal AP activation through its more conventional mechanism—that of stabilizing AP C3 convertase (C3bBb) [101].

Several strains of *N. gonorrhoeae* can regulate the CP and are complement-resistant by virtue of their ability to bind to the CP inhibitor, C4b-binding protein (C4BP)—discussed below. Higher concentrations of anti-LOS mAb 2C7 were required to kill C4BP binding strains compared to C4BP nonbinders. Complement-dependent killing of C4BP binders required properdin and AP activation in addition to CP activation [102].

Deposition of C3 and C4 on Neisseriae

C3, C4, and C5 evolved from a common ancestral protein that contained an internal thioester bond [103]; activated C3 and C4, but not C5, have retained the capacity to form covalent bonds with their targets (Fig. 2A). Autoactivation of the AP occurs because of spontaneous 'tickover' of C3, where hydrolysis of C3 generates a conformationally altered C3 molecule called $C3(H_2O)$ that binds to FB [104]. FB bound to $C3(H_2O)$ is cleaved by factor D (FD) to generate C3 $(H_2O)Bb$, which has C3 convertase activity and cleaves the C3a fragment from the N terminus of the α chain of C3 to yield C3b. Cleavage of C3 results in a conformational change in the molecule and exposure of its internal thioester bond. The calculated half-life of the thioester of this metastable C3b molecule is $\sim$ 60 μs [105]. Within this short period, the nascent C3b must find a suitable electron donor in the form of an -OH on biological surfaces to form a covalent ester bond; failure to do so will result in reaction of C3b with a water molecule, and inactive C3b will remain in solution. The labile nature of activated C3b ensures that C3b is deposited only on targets proximate to the site of complement activation, thereby preventing indiscriminate damage to distal tissue.

C3b binds covalently to hydroxyl groups in carbohydrates and serine, threonine and tyrosine in proteins. Sugars differ in their ability to react with C3b—for example, ribose reacts about 12- and 2-fold more effectively than inositol and glucose, respectively [106]. Further, the location of -OH and steric factors affects C3b binding to carbohydrates. 6-OH groups and terminal (rather than internal) -OH residues or branches of polysaccharides on the surface of microbes preferentially bind C3b [106]. Tyrosine reacts with the

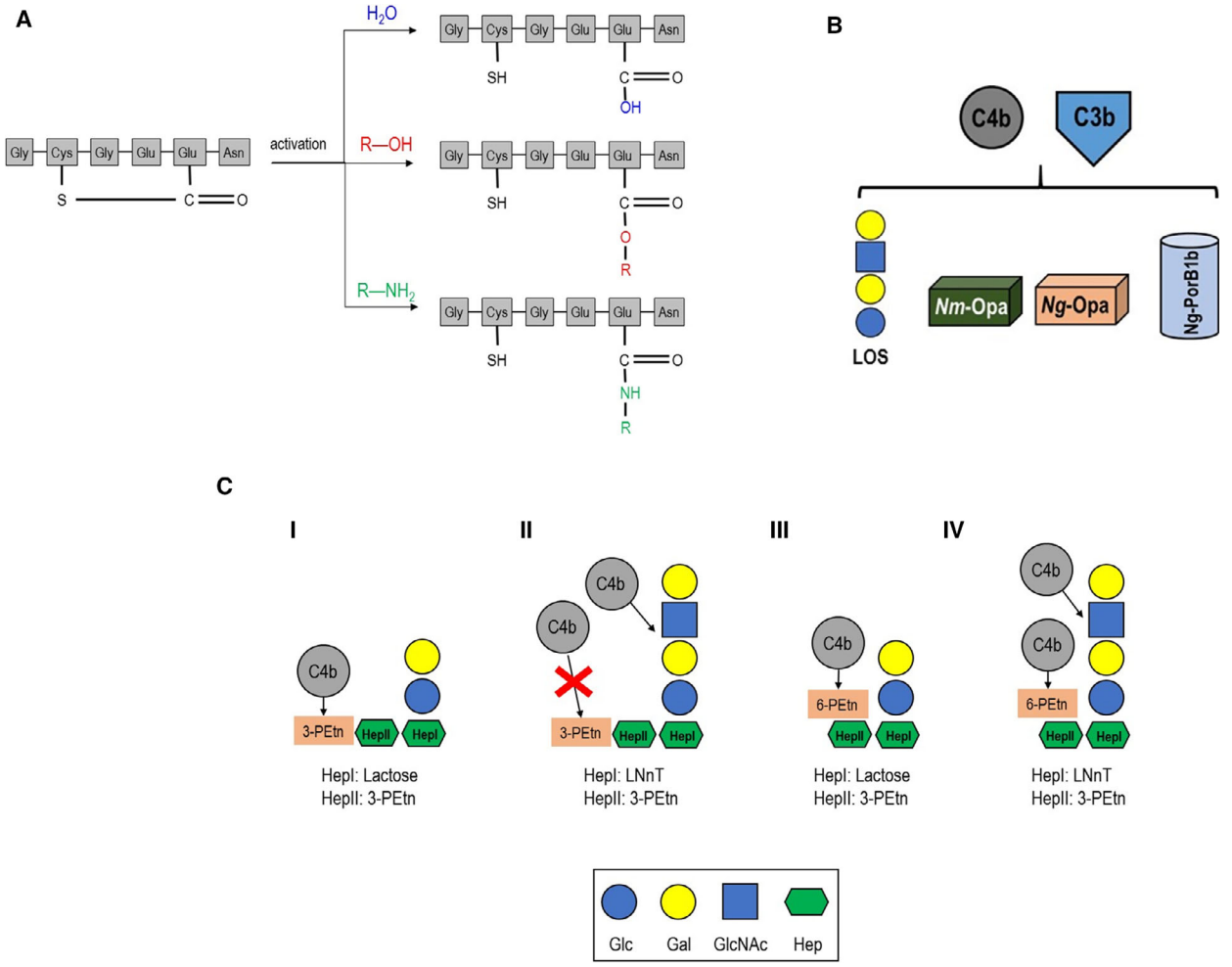


Fig. 2. C3 and C4 fragment deposition on Neisseriae. (A) The thioester bond of C3. During activation, C3a is released from the amino terminus of the α-chain of C3. The exposed internal thioester bond becomes accessible to nucleophilic attack and can react with water or available hydroxyl or amine groups on cell surfaces. Analogous reactions occur with C4. Together, these reactions involving C3 and C4 are responsible for covalently linking complement deposition to the cell surface. Asn, asparagine; Cys, cysteine; Glu, glutamic acid; Gly, glycine; Met, methionine. (B) Targets for C3b and C4b on *Neisseria*. LOS, Opacity proteins (Opa) and Porin B1b (PorB1b) are the major targets for C3b and C4b on *N. gonorrhoeae* and *N. meningitidis*. (C) LOS glycan extensions from HepI and the position of phosphoethanolamine (PEtn) substitutions on HepII modulate C4b deposition on the LOS of *N. meningitidis*. When HepI is substituted with lactose, PEtn at the 3-position of HepII is accessible and forms an amide bond with C4b (i). Longer (LNnT) glycan extension from HepI prevents C4b from accessing 3-PEtn; C4b is deposited on a HepI glycan through an ester bond (ii). When PEtn is present at the 6-position, it can form an amide bond with C4b even when HepI expresses longer (LNnT) glycan extension (iv). In this instance, C4b is also deposited on HepI glycans *via* an ester linkage. C4b preferentially binds to 6-PEtn when HepI glycan is truncated (eg, only lactose) (iii). (Fig. 2C is adapted from Ram et al. J Biol Chem. 2003 Dec 19;278nn:50853-62.)

metastable thioester bond of C3b 11- and 47-fold better than threonine and serine, respectively, and about 50-fold better than to most carbohydrates [107]. Thus, the site of C3b deposition on a microbe would depend on the availability and exposure of reactive hydroxyl groups. Once deposited on a surface, C3b can then bind to factor B, which is cleaved by FD to generate more C3 convertases and thus set into motion the positive feedback amplification loop, a unique feature of the alternative pathway.

Humans possess two distinct isoforms of C4, called C4A and C4B (distinct from the C4 fragments generated by CP C3 convertases, called C4a and C4b) that are encoded by *C4A* and *C4B* which are arranged in tandem [108]. Deletions and duplications result in varying copy numbers of *C4A* and *C4B* across individuals; about

50% of Caucasians possess 2 copies each of *C4A* and *C4B*. When activated by CP C3 convertases, C4A and C4B react with -NH2 and -OH groups to form amide or ester linkages with their targets, respectively [109–111]. Differences in the binding properties of C4A (amide) and C4B (ester) may have important functional consequences. C4B is believed to possess greater hemolytic activity than C4A [112]. On the other hand, C4A binds to complement receptor 1 (CR1) more efficiently and may play an important role in clearing immune complexes from the bloodstream [113,114]; persons deficient in or who possess lower copy numbers of the C4A gene have a higher incidence of autoimmune diseases [11,115].

C3 fragment deposition on gonococci and meningococci revealed that most of the C3b associated with Neisseriae was rapidly cleaved to iC3b as early as 5 min [116,117]. Although iC3b is hemolytically inactive, it is an important ligand for complement receptor 3 (CR3) that mediates phagocytosis. Truncation of the glycans on HepI LOS of a group B meningococcal isolate, which resulted in only a Glc expressed from HepI, was associated with increased IgG, IgM, and C3b deposition [117]. Gonococcal LOS, Opa, and PorB1b (an allele of PorB) were described as the major targets for C3b and C4b deposition on Neisseriae [118] (Fig. 2B). All Opa-expressing mutants (Opa's A, B, C, D, E, and I on strain FA1090) bound C4b through amide bonds [118]. Loops 4 and 5 in PorB1b of strain MS11 contained a putative site for deposition of amide-linked C4b. Studies using meningococcal LOS mutants showed that C4Bb and C4Ab formed ester and amide bonds, respectively, with LOS [119]. Amide linkages were formed with phosphoethanolamine (PEtn) at the 6-position of HepII, which was accessible only when 2 or fewer glycans were present on HepI [119] (summarized in Fig. 2C).

Meningococcal capsular polysaccharides have traditionally been associated with increased resistance to serum by virtue of their ability to limit complement activation. Most studies have used group B and group C isolates. An examination of alternative pathway activation on each of the five major meningococcal groups (A, B, C, W, and Y) showed that groups W and Y capsules activated the AP and themselves bound C3 fragments (discussed below and Fig. 3) [120].

Complement inhibition on Neisseriae

N. meningitidis capsular polysaccharide

Capsular polysaccharide is the major virulence factor of meningococci. With a few exceptions [121–124], almost every isolate recovered from the bloodstream or the cerebrospinal fluid of patients is encapsulated. Nongroupable (unencapsulated) isolates that often inhabit the nasopharynx are sensitive to killing by low concentrations of normal human serum (NHS) [125]. Even unencapsulated isolates that are sometimes recovered from the bloodstream of immunocompromised persons and individuals on eculizumab treatment are sensitive to killing by NHS [124,126].

The capsules of group B and group C meningococci are homopolymers of sialic acid (N-acetylneuraminic acid, or Neu5Ac) that are α2-8- or α2-9-linked, respectively. In addition to Neu5Ac, the group W and Y capsules also contain galactose (Gal) and glucose (Glc), respectively (Neu5Acα2-6-Gal- and Neu5Acα2-6-Glc-). The group A meningococcal capsule contains polymers of *O*-acetylated (α1-6)-linked *N*-acetyl-D-mannosamine-1-phosphate (Fig. 3). The A, C, W, and Y polysaccharides can also be *O*-acetylated.

It has long been recognized that sialic acid on erythrocytes inhibits the AP by promoting the interaction between C3b and FH [127]. Early studies that focused mainly on groups B and C meningococci showed that capsule contributed to complement resistance [128] and was antiphagocytic [129]. However, groups B, C, W, and Y meningococci all synthesize

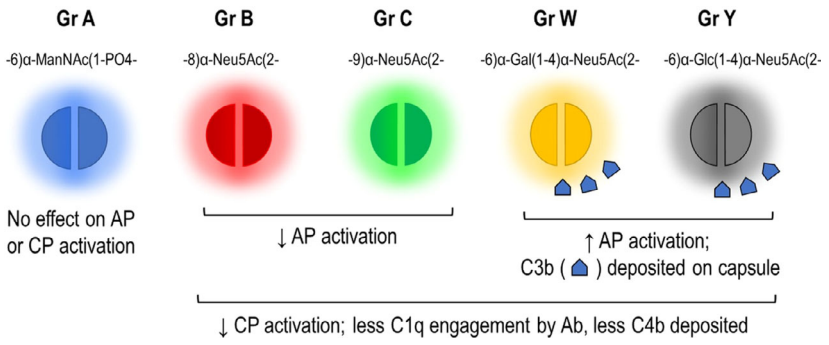


Fig. 3. Schematic showing mechanisms of complement inhibition mediated by capsular polysaccharide of the five major serogroups of *N. meningitidis*.

cytidine monophospho-N-acetylneuraminic acid (CMP-Neu5Ac) and therefore can also sialylate the LNnT (Gal-GlcNAc-Gal-Glc) and P^K antigen-like (Gal-Gal-Glc) structures expressed by LOS. Studies that used neuraminidase showed that sialic acid inhibited complement activation; however, they did not distinguish between the roles of Neu5Ac in capsule versus LOS [57]. Isogenic groups B and C meningococcal mutants that differed only in LOS sialic acid showed that capsule was the major driver of complement resistance [130]. Consistently, meningococcal LOS sialylation alone (in the absence of capsule) did not confer complement resistance, but its presence in encapsulated strains maximized resistance to high concentrations of complement [131]. Both LOS and capsule sialic acids contribute to virulence in the infant rat model of bacteremia [132].

Capsular polysaccharides regulate complement in a serogroup-specific manner (Fig. 3). Expression of group B and C capsules is accompanied by less C3b deposition, while the group A capsule does not limit or enhance AP activation [120]. Greater AP activation was observed on encapsulated group W and Y strains incubated in sera with only an active AP as compared to isogenic unencapsulated mutants [120]. This observation was rather unexpected because groups W and Y capsules both contain Neu5Ac which, as discussed above, is usually associated with complement inhibition. However, the groups W and Y capsules themselves bound C3b. Only capsular polysaccharide associated with the bacterial surface, but not soluble capsules, activated complement and bound C3b, which was consistent with other studies that showed that only particulate, but not soluble glycans activated the AP [133,134]. Despite increased AP activation, expression of groups W and Y capsules is associated with increased resistance to killing by NHS, probably because of their ability to inhibit the CP—the pathway that initiates and drives killing of Neisseriae—as discussed below.

The effects of meningococcal capsule expression on CP activation were studied on mutants engineered to express similar amounts of FHbp [135]. Normalizing antibody binding permitted evaluation of the effects of capsule expression on C1q engagement by antibody, activation of C4 by antibody-bound C1 complex, and C4b deposition. B, C, W, and Y capsules all interfered with C1q binding to the anti-FHbp mAb and subsequent C4b deposition. Expression of group A capsule did not block C1q engagement by anti-FHbp [135]. A group C strain that was highly resistant to killing by immune (vaccine) serum was found to be 'hyper-encapsulated' as a result of an IS 1301 sequence insertion that increased capsule synthesis and transport, providing an additional evidence for capsule inhibiting the CP [136].

Lipooligosaccharide (LOS) sialylation

Fifty years ago, Ward et al showed that all gonococci recovered directly from male urethras were fully resistant to complement-dependent killing by NHS [137]. However, several strains lost the ability to resist NHS after even a single passage on artificial media [137]. Almost two decades later, Harry Smith and his colleagues showed that gonococci scavenged CMP-Neu5Ac from its host to sialylate its LOS [138–142]. The addition of CMP-Neu5Ac to growth media restores complement resistance to serum-sensitive (unsialylated) strains. The transfer of sialic acid to LOS is mediated by an enzyme called LOS sialyltransferase (Lst) located in the outer membrane of *N. gonorrhoeae* [143]. Meningococci also express Lst and sialylate their LOS. Similar to gonococci, group A meningococcal strains require an exogenous source of CMP-Neu5Ac, whereas groups B, C, W, and Y can synthesize CMP-Neu5Ac and therefore do not rely on their host for the sialic acid donor molecule [144].

The two LOS glycan extensions from HepI on *N. gonorrhoeae* and *N. meningitidis* that can be sialylated are as follows: LNnT (Gal-GlcNAc-Gal-Glc) and the P^K-like antigen, also known as the L1 immunotype in meningococci (Gal-Gal-Glc) [145,146]. Recently, lactose extensions from HepII of *N. gonorrhoeae* LOS were also shown to accept sialic acid [147]. Meningococci do not add glycans beyond the proximal Glc on HepII and sialylation of HepII LOS does not occur. Neu5Ac is linked to LNnT through an α2-3 linkage, while the terminal Gal residues of the P^K and lactose are substituted with α2-6-linked Neu5Ac (Fig. 4).

LOS sialylation inhibits all three pathways of complement. Binding of IgG present in NHS is inhibited by gonococcal LOS sialylation, which reduces C4b deposition and enhances serum resistance [148]. Inhibition of IgG binding is antigen-specific—for example, gonococcal LNnT sialylation reduces the binding of IgG mAbs to PorB, but not mAbs to Opacity protein (Opa) [149]. Both meningococcal and gonococcal LNnT LOS sialylation also inhibit binding of purified MBL, which leads to reduced C4b deposition [79,82,83]. Sialylation of lactose extensions from HepII of *N. gonorrhoeae* did not reduce binding of an anti-LOS mAb, but inhibited complement-dependent killing *in vitro* [147]. Given that LOS is a target for both C4b and C3b [73,118], it is reasonable to speculate that

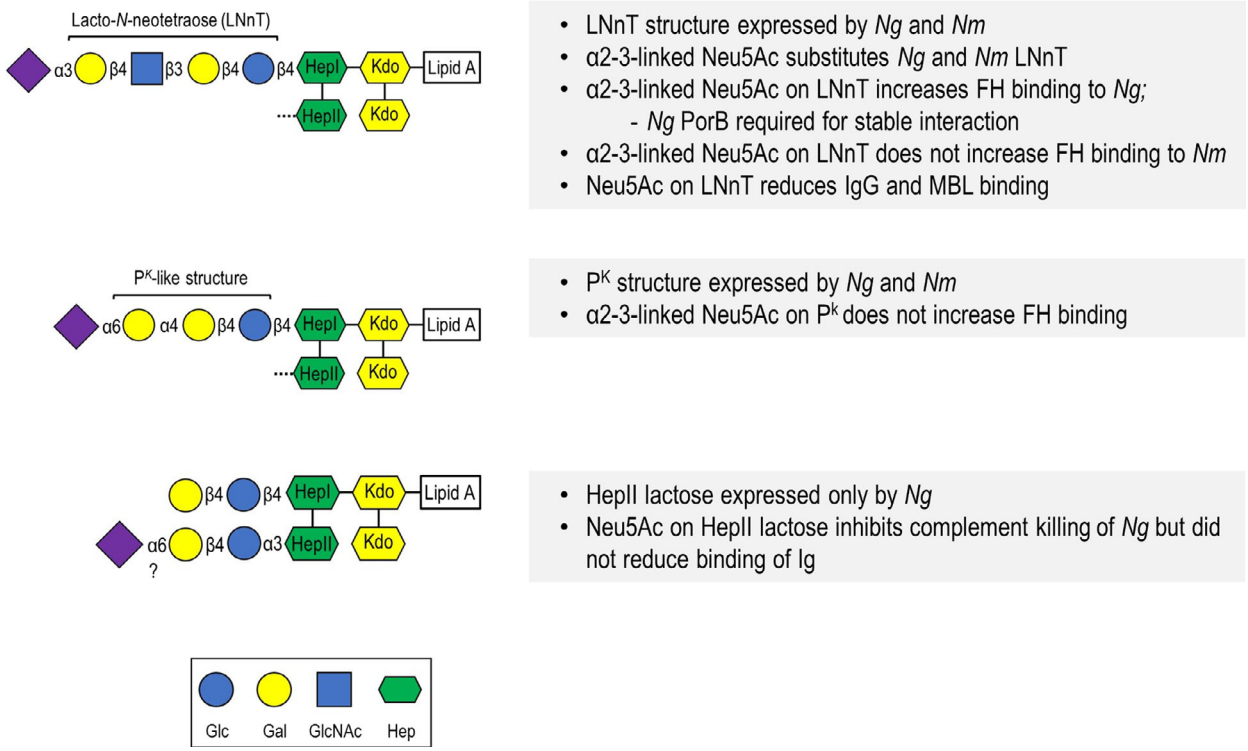


Fig. 4. Inhibition of complement by LOS sialic acid. The three glycoforms of Neisserial LOS that can be sialylated include the following: lacto-N-neotetraose (LNnT) from HepI (*top panel*), the P^K-like structure (*middle panel*), and lactose from HepII (*lower panel*). HepII lactose is expressed only by gonococci. Mechanisms of complement regulation that have been described for each are indicated alongside the schematic.

sialylation obscures targets for these complement activation products.

Complement activation can damage host tissues and a complex regulatory network exists to balance between activating complement on nonself 'foreign' surfaces and inhibiting complement on self surfaces. Sialylation of host surfaces enhances FH binding and helps discriminate these surfaces as 'self'. Kajander and colleagues showed that FH domains 19 and 20 interacted with (erroneously) deposited C3d and surface-associated Neu5Ac to mediate a stable FH-cell interaction and limit further complement activation. Complement activation is favored on 'foreign' surfaces that lack FH-binding sialic acids or glycosaminoglycans ('self–nonself discrimination') [150]. Only certain glycosaminoglycans, such as dextran sulfate, heparin, carrageenan, and chondroitin sulfate A, but not others including chondroitin sulfate C, hyaluronic acid, and colominic acid (α2-8-linked polysialic acid—identical to group B meningococcal polysaccharide), enhance the interaction between FH- and surface-associated C3 fragments [151,152]. The structural basis for the interaction of FH domain 20 with Neu5Ac revealed that FH-Neu5Ac interactions are restricted to α2-3-linked Neu5Ac; no binding is seen to α2-6-, α2-8-, or α2-9-linked Neu5Ac [153]. Consistent with these observations, α2-3-linked Neu5Ac to LNnT enhances FH binding (discussed further in the section on FH recruitment; Fig. 4) and immune evasion [146,147]. Of note, α2-6-linked Neu5Ac to P^K LOS and capsular sialic acids (α2-8- or α2-9-linked Neu5Ac) do not enhance FH binding. Factor H binding to gonococci with α2-3-linked Neu5Ac to LNnT helps explain the observations of Ward [137] and Smith [142]. The multifaceted mechanisms whereby LOS Neu5Ac inhibits complement are summarized in Fig. 4.

It is important to note that LOS glycans and PEtn substitutions (independently of Neu5Ac) also modulate resistance to complement. Naturally occurring *N. meningitidis* and *N. gonorrhoeae* express at least a lactose from HepI because the genes that encode the LOS glycosyltransferases LgtF and LgtE that add the proximal Glc and the subsequent Gal residues to HepI are constitutively expressed (i.e., are not phase variable). Mutants that lack HepI glycan extensions (e.g., *lgtF* or phosphoglucomutase (*pgm*) mutants) are more serum susceptible than their parent isolates [131,154]. The proximal Glc on HepI is important for maximal

binding of C4BP, a key CP inhibitor (discussed further below) [154]. Mutant gonococci and meningococci that express only a proximal Glc from HepI remain serum resistant, but tend to be more serum sensitive than their wild-type parent strains [131,154].

In gonococci, expression of a terminal GalNAc residue beyond LNnT that is controlled by the phase-variable *lgtD* enhances serum sensitivity [155] because GalNAc is the target for natural IgM antibodies that are bactericidal [156]. Phase variation of *lgtD* such that a terminal GalNAc is not expressed on LOS represents a mechanism of complement evasion. In some instances, expression of only lactose from HepI may render a strain more resistant than the parent isolate that expresses LNnT, which may be related to enhanced binding of FH (discussed below). Finally, loss of the 4′ PEtn residue from lipid A also enhances serum sensitivity. Lipid A PEtn expression is necessary for maximal C4BP and FH binding [157], as discussed below.

Cleavage of C3 by meningococcal NalP

Several bacteria secrete proteases that degrade various complement components and enable them to evade killing by complement. Thus far, only one complement component cleaving protease, called NalP [158,159], has been identified in *N. meningitidis*, but not *N. gonorrhoeae*. NalP is a phase-variable autotransporter with subtilisin-like serine protease activity that is involved in autoproteolytic processing, resulting in secretion of the NalP passenger domain into the bacterial supernatant. Both the full-length membrane-anchored protein and the smaller passenger domain that is released by autocatalytic processing can cleave C3 [160]. Consistent with humans being the exclusive natural reservoir of meningococci and analogous to other host-restricted Neisserial pathogenic processes, NalP protease activity is restricted to human C3; cleavage of rabbit or mouse C3 was not observed [160].

Recruitment of complement inhibitors

A common strategy used by several pathogens to evade complement-mediated killing is binding of host complement inhibitors such as FH, C4BP, and/or vitronectin, which results in inhibition of complement on the microbial surface (reviewed in refs [4,161]. The numerous mechanisms Neisseriae have evolved to scavenge complement inhibitors, and escape complement activation will be discussed next (summarized in Fig. 5).

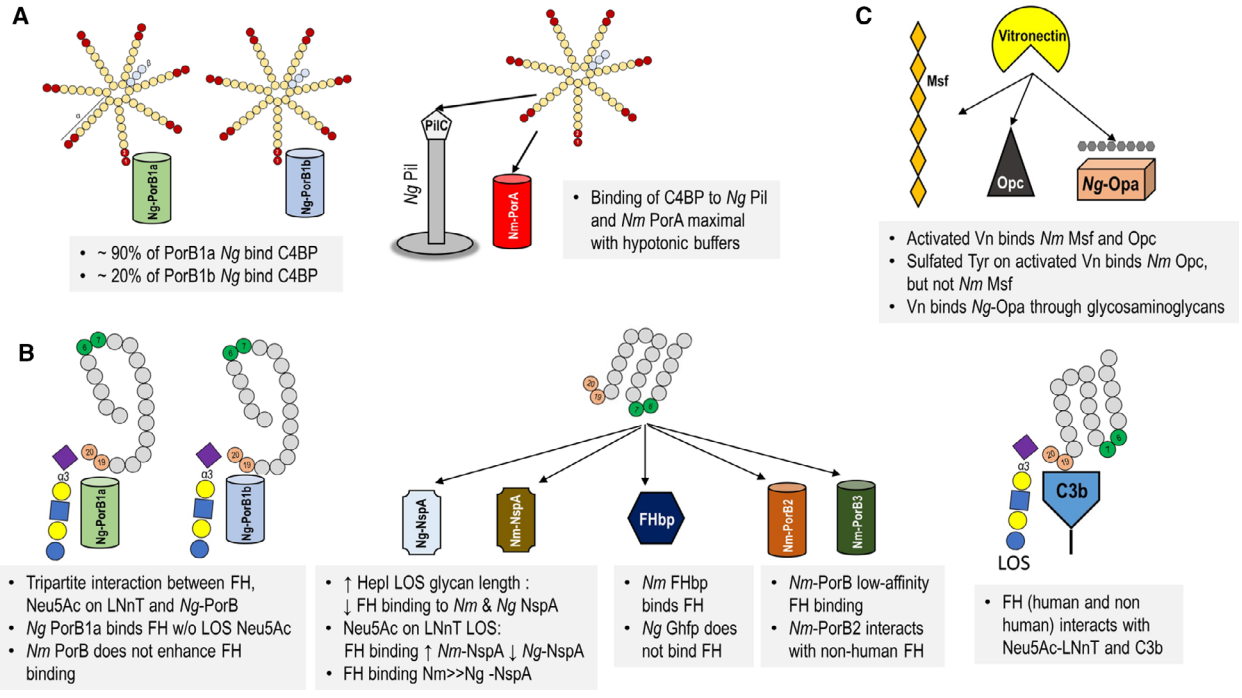


Fig. 5. Recruitment of the complement inhibitors, FH, C4b-binding protein (C4BP), and vitronectin by *Neisseria*. A, B, and C schematically show C4BP, FH, and vitronectin recruitment by Neisseriae, respectively. Salient features of each of the interactions are indicated in the boxes beneath each schematic.

C4b-binding protein (C4BP)

C4BP inhibits the CP and is composed of seven identical α chains, each of which comprises 7 complement control protein (CCP) domains and a β chain that is made up of 3 CCP domains [162]. C4BP inhibits the CP by: (a) acting as a cofactor for FI-mediated cleavage of C4b to C4c and C4d and (b) irreversibly dissociating the CP C3 convertase, C4bC2a [163,164]. *N. gonorrhoeae* bind to C4BP through their most abundant outer membrane protein, Porin B (PorB). PorB exists as a trimer and each subunit comprises 16 transmembrane segments and 8 extracellular loops [165]. Gonococcal *porB* exists as two alleles—*porB1a* and *porB1b* that possess about 80% homology at the nucleotide level. Each gonococcal strain expresses a single *porB* allele. Strains that cause disseminated disease often express PorB1a (also known as PorB.1A or Por1A), while the majority of strains that cause local disease express PorB1b (PorB.1B or Por1B). About 90% of PorB1a and about 20% of PorB1b strains bind C4BP [166]. A strong positive correlation exists between the ability of gonococci (with unsialylated LOS) to bind C4BP and their ability to resist complement-dependent killing by NHS [166,167].

How C4BP interacts with PorB remains unclear and may involve a complex conformational structure. Using a large panel of PorB mutants derived from strain FA1090 (a C4BP binder that expresses PorB1b) that included deletions of segments of individual loops or changing multiple sequential amino acids to alanine, Chen and Seifert concluded that loops 4 through 7 were important, as was a sequence of the transmembrane region just downstream of loop 6 [168]. A previous study that used PorB hybrids derived from PorB1b strains MS11 (binds C4BP) and F62 (C4BP nonbinder) suggested that loops 5 and 7 in PorB1b were critical for C4BP binding [167]. Loop 1 of PorB1a strain FA19 was essential for C4BP binding [167]. LOS structure also factors into C4BP-PorB interactions on whole bacteria—loss of lipid A PEtn (*eptA* [formerly called *lptA*] deletion) [157] or loss of glycan from HepI (*lgtF* deletion) both adversely impact C4BP binding and result in serum sensitivity [154]. The binding site for PorB1a and PorB1b in C4BP resides in C4BP CCP domains 1 and 2 [167].

Pili on *N. gonorrhoeae* also interacts with C4BP, mainly through the N terminus of the PilC component [169]. Pili are key bacterial adhesins, but C4BP binding to PilC did not interfere with adherence of gonococci to epithelial cells [169]. The role of the PilC-C4BP interaction on serum resistance was not tested and remains unclear.

Neisseria meningitidis can express two distinct porin molecules, PorA and PorB. PorB exists as two alleles, PorB2 or PorB3. Meningococci recruit C4BP through PorA [170]. As with *N. gonorrhoeae* PilC, maximal binding of C4BP to PorA occurred under hypotonic conditions. Expression of capsular polysaccharide inhibited binding of C4BP to *N. meningitidis*. Blocking C4BP binding to meningococci using Fab fragments of an anti-C4BP mAb increased killing, demonstrating function of C4BP bound on the bacterial surface [170]. The interactions between C4BP and *Neisseria* are shown schematically in Fig. 5A.

Natural infections with *N. gonorrhoeae* and *N. meningitidis* are restricted to humans. Several factors contribute to the host restriction of Neisserial infections, including their ability to scavenge iron and zinc from only human sources [171] and their ability to bind only to human cell receptors (e.g., CEACAMs) (reviewed in ref. [172]. Complement evasion by *N. gonorrhoeae* and *N. meningitidis* is also restricted to humans, in part because they bind only to human C4BP and FH [173,174]. An exception is the ability of select PorB1b gonococci that bind to human C4BP to also bind chimpanzee C4BP and resist killing by chimp complement [173]. It is worth noting that two gonococcal strains that were used in 1970s to successfully infect chimpanzees both expressed PorB1b, whereas a PorB1a strain consistently failed to infect chimpanzees [175]. The moratorium on chimpanzee experiments will leave unanswered the question of whether the successful urethral colonization by PorB1b gonococci relates to their ability to bind chimpanzee C4BP.

Factor H binding

Factor H is a key inhibitor of the AP. Analogous to C4BP, FH possesses cofactor activity (enables FI to cleave C3b to iC3b) and decay-accelerating activity (cleaves C3bBb irreversibly) [176,177]. FH comprises 20 CCP domains arranged as a single chain [178]. *N. gonorrhoeae* and *N. meningitidis* have evolved several redundant and distinct mechanisms to bind FH (summarized in Fig. 5B).

Enhancement of FH binding to gonococci with LNnT sialylation (discussed above) specifically requires gonococcal PorB; allelic replacement of gonococcal PorB with meningococcal PorB does not enhance FH binding when measured by flow cytometry [179]. These data suggest a stable tripartite interaction is formed between LOS Neu5Ac, gonococcal PorB, and FH C-terminal CCP domains 18 through 20 of FH.

The C terminus of both human and nonhuman FH can bind simultaneously to C3b fragments deposited

on meningococci and Neu5Ac on LNnT LOS [180] in a manner analogous to FH interactions with glycosaminoglycans/sialic acid and C3 fragments (C3b and C3d) on human cells [150]. Binding of FH directly to bacteria usually occurs within seconds—well before C3b deposition occurs—and thus, very effectively prevents AP C3 convertase formation. FH that binds to C3b and Neu5Ac is less effective in limiting complement activation because a portion of deposited C3b will form C3 convertases (as opposed to binding FH) that will cause bacterial damage.

Several Neisserial proteins that bind FH have been described. Most gonococcal strains that express PorB1a—the allotype often associated with disseminated gonococcal infection—bind FH directly and independently of LOS sialylation [181]. Meningococcal PorB2 (the ortholog of gonococcal PorB1b) and select meningococcal PorB3 (the ortholog of PorB.1a) also bind FH [182,183]. Although binding of FH to meningococcal PorB in most instances is too weak to be detected by flow cytometry, the functional consequence of FH binding (C3 deposition or resistance to killing by complement) is readily apparent [182,183].

Neisserial surface protein A (NspA), a transmembrane protein that has 4 short extracellular loops and is expressed by gonococci and meningococci [184,185], binds human FH [186]. Stronger FH binding is seen to meningococcal NspA than to gonococcal NspA [187]. Domains 6 and 7 in FH interact with loops 2 and 3 of NspA [187]. LOS structure has a profound effect on the strength of FH binding to NspA, which may be the result of limited surface exposure of the NspA loops [186]. Binding to NspA increases as LOS length decreases. However, Neu5Ac substituted LNnT has opposing effects on FH binding to NspA on *N. meningitidis* and *N. gonorrhoeae*; whereas sialylation increases FH binding to *N. meningitidis* [186], it reduces FH binding to NspA on *N. gonorrhoeae* [187]. As with FH-PorB interactions on *N. meningitidis*, function of FH bound to NspA on *N. gonorrhoeae* is apparent even when binding is weak [187]. The high concentrations of FH in serum ($\sim 500\ \mu g{\cdot}mL^{-1}$) maintain an 'on' rate sufficient to inhibit complement despite low-affinity interactions.

Meningococcal FHbp is a high-affinity ligand for human FH [188,189]. FHbp is a lipoprotein and is surface expressed on meningococci, but the gonococcal FHbp homolog, Ghfp, lacks a signal sequence and a lipid modification motif and the protein is not expressed on the bacterial surface. The reader is referred to the review by Rappuoli and Pizza (in preparation) for a detailed review on FH-FHbp interactions and its role in complement resistance.

FH binding to NspA, FHbp, and gonococcal PorB is also human-specific [174,187,188,190]. Binding of FH to microbial ligands may have implications for vaccine efficacy. Binding of FH to FHbp may skew antibody response to epitopes not obscured by the relatively large interacting surface areas between FHbp and domains 6 and 7 of FH. Bactericidal responses elicited by FHbp may be improved by engineering FHbp mutants that do not bind human FH [191–194]. An exception to the human-specific binding of FH to meningococcal ligands is PorB2, which interacts with rat and rabbit FH to limit AP activation and may an explanation for greater virulence of PorB2 compared to PorB3-expressing meningococci in the infant rat model of bacteremia [195].

Vitronectin

Vitronectin is a multifunctional glycoprotein; one of its functions is the prevention of MAC assembly [196–198]. Two meningococcal surface proteins, Opc and Meningococcal surface fibril, Msf (also known as NhhA and Hsf), bind to the activated (partially unfolded) form of vitronectin [199–201]. The opacity protein, Opc, is a phase-variable 10-stranded β-barrel with 5 extracellular loops [202,203]. Msf is an adhesin predicted to exist as trimeric fibrils of ~ 45 nm [201]. Sulfated tyrosines on the activated form of vitronectin bind to Opc, which then permits the cell-binding RGD domain of vitronectin to bind to human brain endothelial cells and facilitate bacterial adhesion to cells [199]. Vitronectin–Opc interactions also decrease MAC deposition and enhance resistance to complement-mediated killing. Consistent with the MAC-inhibiting function of vitronectin, expression of Opc and Msf both reduce MAC deposition but not deposition of C3 [201]. Expression of Opc and Msf varies among meningococcal strains, and expression of both proteins simultaneously increases serum resistance relative to the expression of either protein independently [201]. Bovine and murine vitronectin both possess conserved sulfated tyrosine residues and interact with Opc [199]. This contrasts with the largely human-specific binding of FH and C4BP to Neisseriae.

Opacity proteins (Opa) expressed by Neisseriae are important for mediating adhesion of bacteria to epithelial cells. Gonococcal opacity protein A (OpaA) binds sulfated polysaccharides such as heparin and dextran sulfate, which in turn can recruit vitronectin [204]. While 'indirect' binding of vitronectin facilitates

attachment and entry of gonococci into epithelial cells [205], its role in complement evasion remains undefined. Other Opa variants such as Opa's E and F may also contribute to complement resistance [206]. Neisserial interactions with vitronectin are shown schematically in Fig. 5C.

As its name suggests, Neisserial heparin binding antigen (NHBA) on *N. meningitidis* also recruits heparin, which enhances serum resistance of unencapsulated meningococci [207]. A recent report suggested that NHBA expressed by gonococci also contributed directly to serum resistance (independent of heparin binding) [208]. The mechanism(s) of complement resistance mediated by gonococcal Opa and NHBA remain to be elucidated.

Blocking antibodies

In some instances, antibodies elicited by natural infection, colonization, or even immunization may block complement-dependent killing of gram-negative bacteria by otherwise bactericidal antibodies. This phenomenon, termed 'blocking', was described by Pfeiffer and Isaaeff as far back as 1894 [209] and subsequently has been described for several pathogens including *Brucella* spp [210,211], *Pseudomonas aeruginosa* [212,213], nontyphoidal Salmonella [214], *E. coli* [215], *N. gonorrhoeae*, and *N. meningitidis* (discussed below).

Blocking antibodies against *N. gonorrhoeae*

McCutchan et al. [216] showed that strains that caused disseminated gonococcal infection (DGI) were resistant to human serum but killed by normal rabbit serum. Killing by rabbit complement was blocked by the addition of IgG from nonbactericidal human serum. Similarly, IgG as well as $F(ab')_2$ fragments of IgG derived from 7 of 10 nonbactericidal volunteer sera blocked killing of serum resistant isolates by convalescent or immune DGI serum [217]. Most of the blocking activity was directed against reduction-modifiable protein (Rmp; also referred to as protein III, or PIII) [218]. Interestingly, incubating gonococci simultaneously with a mixture of bactericidal and blocking anti-Rmp IgG resulted in decreased killing, but increased C3 and C9 deposition on bacteria, compared to bacteria incubated with bactericidal antibody alone [219]. About one-quarter of C3 associated with gonococci incubated with blocking antibody was deposited on the antibody. Diversion of C3 onto nonbactericidal targets was proposed as a possible mechanism of blocking [219]. Epitope specificity of anti-Rmp may determine its ability to block killing of gonococci. Of six anti-Rmp mAbs studied by Heckels and colleagues, only one mAb directed against the N-terminal cysteine loop showed blocking activity; one mAb was neither killing nor blocking, while the remaining four were bactericidal [220,221].

The presence of anti-Rmp may also increase the risk of acquiring gonorrhea. Commercial sex workers in Nairobi with anti-Rmp were at an increased risk for acquiring gonorrhea (adjusted odds ratio 3.4; 95% confidence interval (1.1–10.4; $P < 0.05$) [222]. Anti-Rmp elicited by vaccination with recombinant *N. gonorrhoeae* Rmp, or by passive transfer of IgG purified from sera of mice immunized with Rmp, reduced the efficacy of an anti-LOS mAb in the mouse vaginal colonization model [76]. In this model, levels of anti-Rmp in the serum and in vaginal secretions correlated inversely with efficacy of the otherwise protective anti-LOS mAb. Measurements of mouse C3 deposited on gonococci recovered from the vaginas of mice showed that the presence of blocking anti-Rmp was associated with reduced C3 compared to mice that were given anti-LOS mAb alone [76]. This finding of reduced C3 deposition on bacteria recovered from mice contrasts with the prior *in vitro* data where blocking Ab enhanced complement deposition on gonococci [219], and may reflect the differences in the systems (*in vitro* vs *in vivo*) and assays used.

Blocking antibodies against *N. meningitidis*

Thomas and Dingle observed that select sera from rabbits or horses immunized with whole meningococci, or from humans recovering from meningococcal disease interfered with the bactericidal activity of complement [223]. Subsequently, Griffiss showed that IgA purified from human sera drawn 12, 27, and 33 days after infection with serogroups B, Y, and C meningococci, respectively, inhibited meningococcal killing by IgG and IgM purified from the same sera [224]. Inhibition depended on the ratio of lytic to blocking antibody and was strain specific. Further, bactericidal IgG was more readily blocked than IgM. Similarly, IgA isolated from sera of rabbits immunized with group C meningococci also demonstrated blocking activity. In a separate study, sera collected from 24 patients at the time of hospitalization lacked bactericidal activity, which was restored upon depletion of IgA [225]. Blocking IgA1 was directed against group C capsular polysaccharide [226]. The lack of bactericidal activity in the sera of certain Sudanese adults despite high amounts of anticapsular IgG was posited to be because of concomitant high concentrations of anticapsular IgA, although blocking by IgA was not

formally demonstrated [227]. Vaccination of a C2-deficient individual elicited antigroup W capsule IgG that limited the amount of C3 deposited on group W capsule and blocked alternative pathway-dependent killing of group W meningococci [18]. Natural antibodies against Galα1-3-Gal residues cross-react with meningococcal Pilin and can also reduce bactericidal activity of human serum [228]. Human sera that contain antibodies directed against the AAEAP pentapeptide repeat motifs present in H.8 (Lip) and Laz can inhibit classical pathway activation (reduce C4b deposition) and block killing by select antibodies, including those directed against meningococcal FHbp [55]. It is worth noting that anti-Rmp mAbs that block killing of gonococci do not appreciably block killing of meningococci [229].

Conclusions and perspectives

The rapid increase in the number of cases and the emergence of multidrug-resistant gonorrhoea globally is alarming, and there is an urgent need to develop safe and effective vaccines and therapeutics against this disease. Despite the availability of effective vaccines against the five major serogroups, meningococcal infections remain a major cause of morbidity and mortality worldwide. The predisposition to invasive Neisserial infections in individuals with congenital defects of complement now extends to pharmacologic blockade of complement, specifically anti-C5 therapy. Of concern are cases of invasive meningococcal disease seen in vaccinated persons on eculizumab therapy. Careful monitoring for Neisserial infections is warranted with the burgeoning of complement inhibitor therapeutics in clinical trials. Further work is needed to define the risk of these infections with inhibitors of specific pathways of complement.

Neisseria gonorrhoeae and *N. meningitidis* have evolved to mimic their human hosts and utilize several sophisticated mechanisms to escape complement, to adapt and reside in various niches in their human hosts. Elucidation of these mechanisms can inform the development of vaccines and therapeutics that target virulence mechanisms. The development and success of FHbp-based group B meningococcal vaccines is one such example [230,231]. Efforts to improve the bactericidal activity of FHbp by eliminating human FH binding have shown promising results in preclinical studies [191,194,232,233]. An understanding of how complement is activated on bacteria and how subversive (blocking) antibodies prevent complement-dependent killing may also help design effective Neisserial vaccines [234]. An impediment to gonococcal vaccine development has been lack of a correlate of protection. Demonstrating the importance of complement at the mucosal surface for the efficacy of a model chimeric antigonococcal LOS mAb [56] coupled with advances in our understanding of how antibodies activate the classical pathway and how complement is activated on Neisseriae should spur the identification of additional epitopes that elicit bactericidal antibodies for vaccine development.

Our knowledge of complement evasion mechanisms may also be utilized to develop new drugs against multidrug-resistant gonorrhea. Targeting FH and C4BP binding, important gonococcal virulence factors, by chimeric proteins that fuse the gonococcal binding domains in FH and C4BP to Fc have demonstrated efficacy *in vitro* and in mouse models [166,235,236]. Analogues of CMP-Neu5Ac (the substrate for gonococcal LOS sialylation), such as CMP-legionaminic acid (CMP-Leg5,7Ac_2), lead to substitution of gonococcal LOS with the sialic acid analogue that counteracts the complement inhibitory function of Neu5Ac and attenuates gonococcal colonization in mice [148], and represents another example of manipulation of a virulence factor to design therapeutics.

Meningococcal and gonococcal disease represents a growing global threat and therapeutic interventions are needed to curb the spread of disease. Our understanding of the complex interplay between the pathogenic *Neisseriae* and the human complement system provides several promising avenues for the development of novel therapies and preventative measures.

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