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Meningococcal disease and the complement system

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Despite considerable advances in the understanding of the pathogenesis of meningococcal disease, this infection remains a major cause of morbidity and mortality globally. The role of the complement system in innate immune defenses against invasive meningococcal disease is well established. Individuals deficient in components of the alternative and terminal complement pathways are highly predisposed to invasive, often recurrent meningococcal infections. Genome-wide analysis studies also point to a central role for complement in disease pathogenesis. Here we review the pathophysiologic events pertinent to the complement system that accompany meningococcal sepsis in humans. Meningococci use several often redundant mechanisms to evade killing by human complement. Capsular polysaccharide and lipooligosaccharide glycan composition play critical roles in complement evasion. Some of the newly described protein vaccine antigens interact with complement components and have sparked considerable research interest.

Introduction

Despite advances that have contributed to our understanding of disease pathogenesis, *Neisseria meningitidis* (*Nm*) remains an important cause of morbidity and mortality globally. The genomes of >1000 isolates of *Nm* have been sequenced and are available to the public. Many aspects of bacterial colonization, cell invasion, and immune evasion have been elucidated as a result of these data. In addition, genome-wide association studies have identified host factors that may contribute to disease susceptibility. In particular, interaction of molecules of the complement system with the meningococcus has proven important in disease pathogenesis and has contributed to the development of newer vaccine formulations. This review highlights the role of the complement system in the pathogenesis of meningococcal disease and identifies gaps in our knowledge that could inform future research in the field.

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Neisseria meningitidis

Microbiology

Nm is a gram-negative diplococcus, whose biochemical characteristics include catalase and oxidase positivity and the ability to ferment glucose and maltose. Almost all invasive isolates of *Nm* express capsular polysaccharide. Based on the chemical composition of its capsule, meningococci are divided into 12 groups (A, B, C, E [formerly called 29E], H, I J, L, W [formerly W135], X, Y, and Z). The majority of invasive infections worldwide are caused by six of these groups—A, B, C, W, X, and Y. Antigenic variability of the porin B (PorB) and PorA molecules expressed define the organisms serotype and serosubtype, respectively. Because of limited availability of typing and subtying monoclonal antibodies, high-throughput gene sequencing is now commonly used to classify meningococci for epidemiologic studies. Akin to all gram-negative bacteria, meningococci possess lipopolysaccharide (LPS). However, because the LPS of *Nm* lacks the O-antigenic repeats seen in common enteric gram-negative bacilli, it is often referred to as lipooligosaccharide (LOS).

Clinical and epidemiological aspects of meningococcal disease

In 1919, Herrick commented of purpura fulminans, the most ominous and dramatic presentation of meningococcal sepsis, "no other infection so quickly slays"—this quote remains true even today despite considerable advances in biomedicine and our understanding of the pathogenesis of sepsis.

In most instances, the meningococcus is a harmless colonizer of the human nasopharynx.[1,2] Reported rates of carriage vary from 5–10% of adolescents and young adults, to >50% in dormitories and army barracks during epidemics. Acquisition of the bacterium results from close contact with carriers, as may occur with overcrowding (socio-economic inequities, during the Hajj pilgrimage, in college dormitories, and in refugee camps), frequenting nightclubs and bars, or from kissing. The rate of secondary cases among close contacts of an index case can be up to 1000 times greater than the rate of disease in that population.

The highest rates of disease occur in infants under 1 y of age. The incidence of disease declines rapidly thereafter. A second, but smaller peak of disease occurs in adolescents and young adults between the ages of 15 and 25 y. Although several factors may contribute to the susceptibility of an individual to meningococcal disease,[3] the ability of an individual to mount a serum bactericidal response against the challenge strain is probably the single most

Table 1. Characteristics of soluble complement proteins

Component	Approx serum conc (μg/ml)	Mol mass (kD)	Structure	No. of genetic loci	Chromosomal assignment
Classical pathway proteins					
C1q	70	459	18 polypeptide chains; 6A, 6B, 6C; A-B and C-C linked by disulfides	3 (A, B, C)	1p34–1p36.3
C1r	34	173	Dimer (A and B chain linked by disulfide bond)	1	12p13
C1s	31	80	Dimer (A and B chain linked by disulfide bond)	1	12p13
C4	600	206	β-α-γ; 1 β-α and 2 α-γ disulfide bonds	2 *(C4A, C4B)*	6p21.3
C2	11–35	100	1 chain	1	6p21.3
Alternative pathway proteins					
Factor B	200	90	1 chain	1	6p21.3
Factor D (adipsin)	1–2	25	1 chain	1	19p13.3
Lectin pathway components					
MBL	1–5	40	Subunit: trimers of identical polypeptides; subunits organized into larger oligomers (n = 2–6)	1	10q11.2–q21
L-ficolin (ficolin-2)	3–4	34	Subunit: trimers of identical polypeptides; subunits organized into larger oligomers	1	9q34
H-ficolin (ficolin-3)	18	35	As with L-ficolin	1	Chr 1
M-ficolin (ficolin-1)	0.04–0.1 (monocytes and PMNs main source)	Not known	As with L-ficolin	1	9q34
MASP-1	4–30	97	Active form consists of heavy and light chains linked by disulfide bond	1	3q27–28
MASP-2	0.02–0.9	83	Active form consists of A and B chains linked by disulfide bond	1	1p36.3–p36.2
MASP-3	2–10	105	Activation splits 105 kD disulfide-linked dimer into A (58 kD) and B (42 kD); B chain is serine protease domain	1	3q27–28
MAp19	Not known	19	Alternatively spliced version of MASP-2; contains first 2 domains and 4 additional C-terminal aas; head-to-tail homodimer		1p36.3–p36.2
C3	1000–1500	190	β-α, linked by disulfide bond	1	19p13.3–p13.2
Terminal complement components					
C5	75	190	β-α, linked by disulfide bond	1	9q34.1
C6	45	100	1 chain	1	5p13
C7	90	95	1 chain	1	5p13
C8	55–80	151	α-γ dimer linked by disulfide, noncovalently associated with β	3	(α,β)1p32; (γ) 9q34.3
C9	60	71	1 chain	1	5p13

important variable that determines the risk of infection and is discussed below.

Asymptomatic colonization of the nasopharynx very rarely leads to invasive disease. A combination of factors that includes the invasive potential of the strain ("hypervirulent" clones) and the lack of immune defenses against the invading strain contribute to development of clinical disease. The ability to evade killing by complement is of paramount importance for a strain

Table 1. Characteristics of soluble complement proteins (continued)

Component	Approx serum conc (μg/ml)	Mol mass (kD)	Structure	No. of genetic loci	Chromosomal assignment
Positive regulators					
Properdin	5–10	55	Cyclic polymers in head-to-tail orientation; dimers:trimers:tetramers in 26:54:20 ratio	1	Xp11.4–p11.23
Negative regulators					
C1 inhibitor	150	104	1 chain; highly glycosylated	1	11q11–q13.1
C4b-binding protein (C4BP)	150–300	~550	7 disulfide-linked α-chains (8 SCRs) linked to β-chain (3 SCRs) via disulfide (major isoform; α7/β1); minor isoforms α7/β0 and α6/β1	2	(α,β) 1q32
Factor H	500	155	1 chain (20 SCRs)	1	1q32
Factor I	35	90	β-γ	1	4q
Vitronectin (S-protein)	500	75 (65 kD proteolytic fragment also seen)	1 chain	1	17q11
Clusterin (SP-40,40; Apolipo-protein J)	100–300	60/80 (predicted/ observed)	Heterodimer linked by 5-disulfide bond motif	1	8p21–p12

to establish disease. Upon entering the bloodstream complement activation and cytokine release trigger an inflammatory response. Activation and dysregulation of the coagulation system results in disseminated intravascular coagulation (DIC) that heralds some of the dreaded manifestations of meningococcemia, such as purpura fulminans or vascular thrombosis.[1,2] The spectrum and severity of disease is varied; some individuals suffer meningitis without evidence of meningococcemia or sepsis, while others may have meningococcemia that may range in severity from mild to severe sepsis. At the mild end of the disease spectrum is a rare manifestation called chronic meningococcemia, which is characterized by recurrent fevers, arthralgias, and polymorphic cutaneous eruptions; positive blood cultures establish the diagnosis.[4,5]

The complement system

The complement system has traditionally been considered a first-line of innate immune defense against invading pathogens. However, over the past several years several additional roles for the complement system have been uncovered including modulation of adaptive immune response, elimination of immune complexes, and apoptotic cells, metabolism, angiogenesis, tissue regeneration, and organogenesis.[6] This review will focus on complement as it relates to innate immune defenses against bacterial pathogens, with an emphasis on the meningococcus. The complement system comprises about 30 fluid phase molecules and several membrane inhibitors of, or receptors for complement components (summarized in **Tables 1 and 2**). Complement activation on surfaces may be initiated through one or more of three pathways—the classical, lectin, or alternative pathways (**Fig. 1**).

Overview of the Complement System

The classical pathway

The classical pathway (CP) is initiated by binding of antibodies to their target antigens. Binding of an antibody to its target results in a conformational change in the hinge region of the Fc domain that enables engagement of the C1 complex (comprises C1q, C1r, and C1s) and subsequent activation of C4. Differences in amino acid sequence and glycosylation patterns in the C_H2 and C_H3 regions of the heavy chains of antibody contribute to differences in complement activation across IgG subclasses (IgG3 > IgG1 > IgG2; IgG4 does not activate complement). A critical density of IgG binding to a surface is required to allow two adjacent Fc regions to engage C1q and initiate the CP. By contrast, the pentameric nature of the IgM molecule allows a single IgM molecule to engage C1q and activate complement. Therefore, on a molar basis IgM is more efficient in initiating complement activation than IgG.

C1 complex binding by antibody induces a conformational change in C1q, which results in autoactivation of C1r. Activated C1r then cleaves and activates C1s[7] and the latter cleaves a 9 kD fragment from the N-terminus of the α-chain of C4, called C4a, which exposes an internal thioester bond in C4 that links the –SH group of a Cys residue with the carboxyl-terminal group of Glu. Nucleophilic attack of the exposed and metastable thioester in the C4b fragment by hydroxyl or amino groups leads to formation of covalent ester or amide bonds, respectively. Gene duplication has resulted in two isoforms of C4, called C4A and C4B (distinct from C4a and C4b, the cleavage products of C4 by C1s). C4A preferentially forms amide linkages with

Table 2. Complement receptors and membrane-bound complement inhibitors

Protein	Characteristics
Complement receptors with complement inhibitory functions	
CR1	Cofactor for factor I cleavage of C3b to iC3b and further to C3d, and C4b to C4d; binds to MBL and C1q; clearance of opsonized pathogens and C3b/C4b associated with immune complexes ("immune adherence")
CD46	Cofactor for factor I cleavage of C3b and C4b; serves as receptor for pathogens such as measles virus and (?) *Ng*
CD55	Accelerates the decay of C3 convertase assembled on cells.
CD59	Inhibits the assembly of membrane attack complex (C9 polymerization)
Complement receptors	
CR2	Binds primarily to C3d and C3dg; part of the CR2/CD19/CD81 complex that mediates B cell responses to antigens linked to C3 fragments
CR3	Ligand for iC3b; phagocytosis
CR4	Binds for C3d/C3dg; function not known
CRIg	Ligand for the β-chain of C3b/iC3b; role for pathogen clearance in mouse model
C1qR	Ligand for C1q; phagocytosis
SIGN-R1	Complement receptor identified as one of the murine homologs of DC-SIGN; binds select pneumococcal polysaccharides and C1q and can activate the classical pathway in Ab-independent manner
Receptors for anaphylatoxins	
C3aR	Binds C3a/C3a des-Arg; vasodilatation; role in liver regeneration
C5aR	Binds C5a/C5a des-Arg; chemotaxis; G protein-coupled receptor; possible modulatory role in airway inflammation; upregulated on cardiac myocytes and contributes to cardiac dysfunction during sepsis.

primary amines on target surfaces[8] and is hemolytically less active than C4B,[9] which forms mainly ester bonds with available –OH groups. The molecular basis for the preferential reactions displayed by C4A and C4B relates to the presence of an Asp or His, respectively, at position 1106.[10] Activation of C1s also cleaves C2 to produce a small fragment called C2b that is released into solution and a larger fragment called C2a that can bind to C4b to form C4b2a, the CP C3 convertase (note that some authorities have suggested calling the smaller C2 fragment C2a to align with the nomenclature of the smaller cleavage fragments of C3, C4, and C5 that all bear the "a" suffix; however, this review will adhere to the traditional nomenclature).

The lectin pathway

Five lectin molecules that can bind to terminal monosaccharides and initiate complement activation have been described and include two collectins (collagen-containing C-type (calcium-dependent) lectins (mannose binding lectin [MBL] and collectin 11) and three ficolins (fibrinogen collagen lectins) (ficolin-1, -2, and -3 [also called M-, L-, and H-ficolin, respectively]).[11] Ficolins contain a fibrinogen-like domain combined with a collagen-like domain and therefore are not classified as collectins. The recognition molecules of the lectin pathway bear structural similarity with C1q and are trimers of three identical polypeptide units, each of which terminates in a calcium-dependent carbohydrate recognition domain. The trimers are organized into higher-order oligomers that resemble a "bouquet".

The carbohydrate recognition domains on MBL bind to a variety of terminal monosaccharides, including mannose, *N*-acetyl-mannosamine, *N*-acetyl-D-glucosamine, fucose, and glucose. Collectin-11 binds preferentially to L-fucose and D-mannose. The ficolins appear to bind preferentially to acetylated sugars such as *N*-acetyl-D-glucosamine. In addition, M-ficolin binds to *N*-acetyl-D-galactosamine and sialoglycans, such as those present in the capsule of *Streptococcus agalactiae*. Ligands for L-ficolin include β-(1'3)-D-glucan, *N*-acetylneuraminic acid, lipoteichoic acid, C-reactive protein, fibrinogen, DNA, and certain corticosteroids, while H-ficolin binds to fucose. These sugars are frequently expressed on microbial surfaces but rarely occur as the terminal unit on human oligosaccharides or glycoconjugates. The expression of select saccharide patterns on microbes provides a mechanism for differentiating nonself from self and for rapidly activating complement.

In humans, MBL is encoded by a single gene that contains four exons. Exon 1 harbors three missense SNPs that result in amino acid changes in the first part of the collagenous region. These SNPs result in Gly54Asp, Gly57Glu, and Arg52Cys changes and are termed the "B", "C", and "D" alleles, respectively. The wild-type MBL molecule is termed "A". The B, C, and D alleles are collectively referred to as the "O" alleles and each of these three SNPs interfere with high-order oligomer formation that is important for MBL function. In addition to the SNPs in exon 1, there are several additional polymorphisms located in the MBL promoter region, some of which influence MBL expression levels. Three relevant polymorphisms are G/C at −550 (termed H/L), C/G at −221 (termed Y/X), and C/T at +4 of the 5′-untranslated portion of *mbl2* (termed P/Q).[12,13] The promoter alleles are found in linkage disequilibrium with the exon 1 SNPs, which results in a limited number of haplotypes. Seven haplotypes have been described: HYPA, LYPA, LYQA, LXPA, HYPD, LYPB, and LYQC.[14] When the A, or wild-type exon, allele is in *cis* with

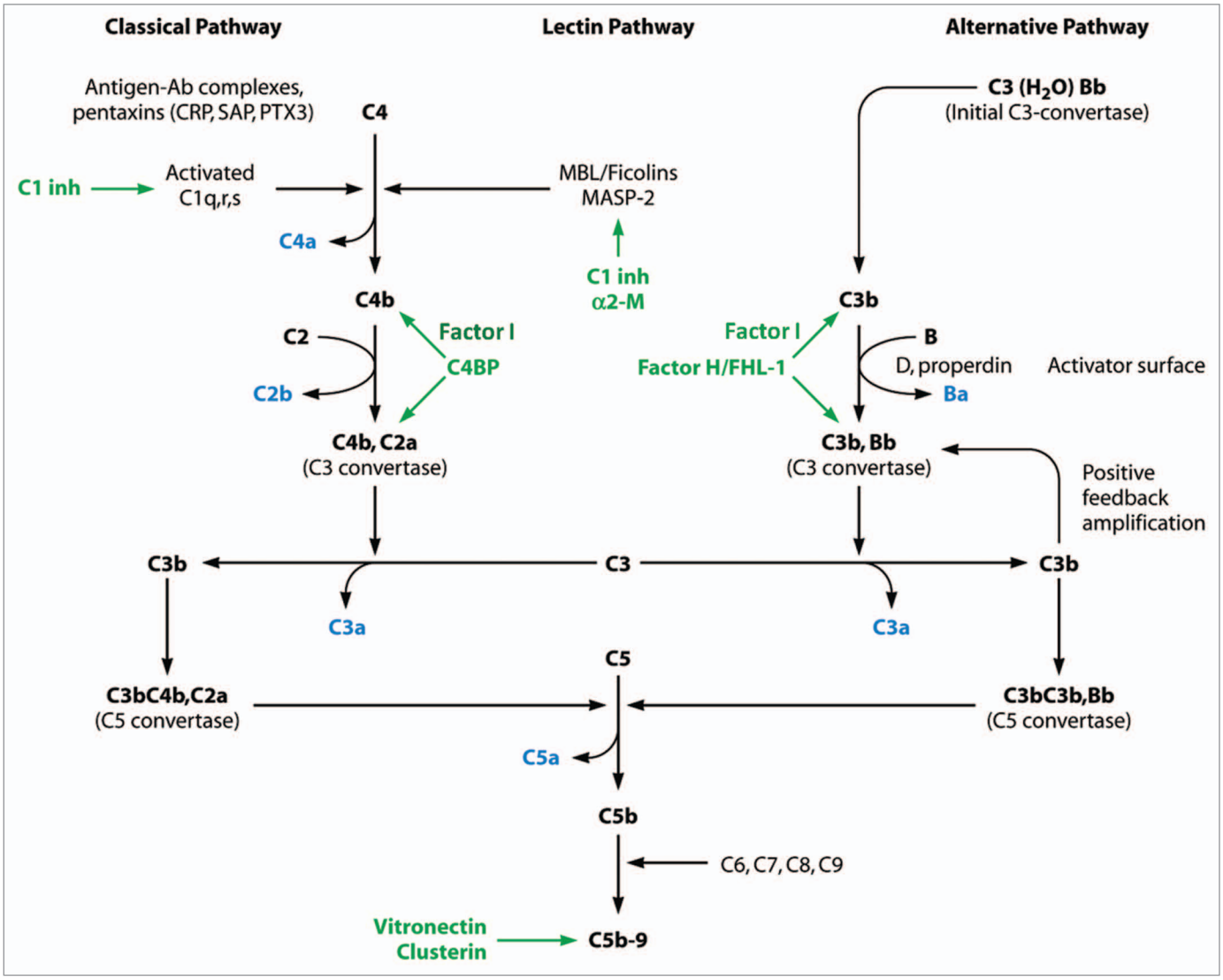

Figure 1. Schematic representing the activation of the complement cascade. The fragments released into solution are indicated in blue font. The key fluid-phase regulators are indicated in green font. CRP, C-reactive protein; SAP, serum amyloid P component; PTX3, pentraxin 3; C1 inh, C1 inhibitor; α2-M, α2-macroglobulin; C4BP, C4b-binding protein; FHL-1, factor H like protein-1. © reference 281.

promoter −550/−221 haplotypes HY, LY, and LX, the MBL concentrations are high, intermediate, and low, respectively. Thus, serum concentrations of MBL reflect the net effects of promoter polymorphisms, structural gene mutations and an interaction between these two factors.

Similar to C1q, the lectins are also associated with serine proteases called MBL-associated serine proteases, or MASPs. The MASPs (MASP1, MASP2, MASP3, and MAp19) are the product of a common ancestral gene shared with C1r and C1s. Recent data suggest that MASP1 transactivates MASP2, analogous to activation of C1r and C1s. Activated MASP2 can then cleave C4 and C2 to generate the CP C3 convertase (C4b,2a).

The alternative pathway

The alternative pathway (AP) of complement is phylogenetically the oldest of the complement pathways. In contrast to the specific protein–protein or protein–carbohydrate interactions that characterize classical and lectin pathway activation, the AP is capable of autoactivation because of a process termed "tickover" of C3. Spontaneous "tickover" of C3 occurs at the rate of ~0.2–0.4% per hour[15] and results in generation of a conformationally altered C3 molecule called C3(H_2O) that is capable of binding factor B. Once factor B associates with C3(H_2O) factor B itself undergoes a conformational change, which renders it susceptible to cleavage by the serine protease factor D, generating Ba and Bb. The Bb fragment remains associated with C3(H_2O) and through its own serine protease domain can cleave 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, akin to that described for C4. The calculated half-life of the thioester of this metastable C3b molecule is ~60 μs,[16,17] which is approximately the time period in which a molecule can diffuse about 300 Å in solution. Within this short period, the nascent C3b must find a suitable electron donor in the form of a –OH or –NH_2 group on a biological surface to

form a covalent ester or amide bond, respectively; failure to do so will result in reaction of C3b with a water molecule and C3b will remain in solution. The labile nature of activated C3b ensures that C3b binding occurs proximate to the site of complement activation thereby preventing indiscriminate tissue damage. C3b that is covalently attached to a surface can bind factor B, which in turn can be cleaved by factor D to produce C3b,Bb, the AP C3 convertase. This sets into motion the positive feedback amplification loop that is a feature unique to the AP. The AP C3 convertase is inherently labile, with a half-life of approximately 90 s. Properdin binding to C3b,Bb stabilizes the complex and prolongs its half-life 5- to 10-fold, thereby providing reaction conditions sufficient for further C3 cleavage and enabling the amplification phase of AP activation.

While complement may be initiated through one or more of the three pathways, the AP can amplify C3 deposited through the classical and lectin pathways, thus potentiating their activity. Antibody may also act synergistically with properdin to facilitate complement activation.[18] Antibody-dependent AP activation depends on the Fab portion of the antibody molecule rather than the Fc fragment that is responsible for CP activation.[18,19] The molecular basis for facilitation is uncertain but may depend on the structure of glycan residues present on IgG. The hydroxyl groups in these moieties may serve as sites for ester bond formation with C3.[19] Moreover, the AP C3 convertase generated on IgG is relatively resistant to inactivation by inhibitory proteins.[19-21]

Hourcade and colleagues proposed a model of activation of the AP where properdin acted as the recognition molecule for surfaces that activated the AP, including zymosan, rabbit erythrocytes, and *Neisseria gonorrhoeae*.[22] This model supported for Pillemer's original claim for the existence of an additional pathway of complement activation,[23] which he called the properdin pathway. Properdin is a positively charged molecule (pI > 9.5) made up of identical subunits, each of which is composed of 6 globular thrombospondin type 1 repeats that associate to form dimers, trimers, and tetramers.[24] Properdin multimers can bind directly to a variety of cell surface molecules, particularly sulfated glycosaminoglycans (GAG), such as heparan sulfate proteoglycans and chondroitin sulfate E. Properdin is synthesized by immune cells, especially those of phagocytic origin. Release of properdin from these cells increases its local concentration and could direct AP activation on specific targets. Properdin bound directly to target cells may bind to C3b and serve as a platform for assembly of AP C3 convertase.[25,26] While properdin is critical for stability of the AP C3 convertase (C3b,Bb) and further C3 amplification on Neisseriae, native, unaggregated properdin does not appear to bind directly to meningococci or gonococci and initiate the AP.[27]

C3—the central component of complement

All three complement pathways converge at the level of C3 activation. C3 is the most abundant complement protein (mean plasma concentrations of ~1 mg/ml) and its cleavage and the stable, covalent linkage of its fragments to target surfaces is a critical outcome of complement activation. The crystal structure of C3 revealed that this molecule is organized into 13 domains.[28] In native C3 the reactive thioester moiety (similar in structure to that described above for C4) that is required for covalent attachment of C3b to target surfaces is sequestered between the thioester domain (TED) and the eighth macroglobulin domain (MG8) where it is protected from nucleophilic attack. Proteolytic cleavage of the C3a fragment from C3 by C3 convertases is accompanied by a remarkable conformational change; the thioester moves ~85 Å from its position in native C3 and becomes fully exposed and available to form a covalent bond as described above.[29] Further processing of C3b is accompanied by additional conformational changes that also actualize novel functions and binding properties. For example, cleavage of C3b generates iC3b, an opsonin and the main ligand for complement receptor 3 (CR3). Cleavage of iC3b creates C3d, which binds to CR2 (CD21) on B cells and triggers ligation of the B cell co-receptor (a complex of CD19, CD21, and CD81) with the B cell receptor to enhance signaling by several orders of magnitude.

Although the complement pathways are often described as separate entities, a germane theme in complement killing of *Nm* is the recruitment of the AP following antibody-initiated complement activation. Several antibodies, including those directed against the vaccine antigen factor H-binding protein (fHbp), require recruitment of the AP and stabilization of the AP C3 convertases by properdin, for maximal killing.[30] The requirement of an intact AP for killing by some antibodies may explain why persons with AP defects are predisposed to *Nm* infections.[31,32] It is important to note that the AP alone (as an example, using C2 depleted serum) does not support direct complement-dependent killing of *Nm*.[33]

The terminal complement pathway

Generation of the membrane attack complex "pore" begins with cleavage of C5. The C3 convertases of the CP and AP (C4b,C2a and C3b,Bb, respectively) can effectively cleave C3, but they exhibit poor activity against C5 (a K_m of approximately 25 μM and a C5 cleavage rate of 0.3 to 1 C5/min at V_{max}).[34] Incorporation of an additional C3b molecule to either of these C3 convertase complexes results in genesis of C5 convertases by changing the K_m for C5 by over 1000-fold from far above physiological C5 concentrations to well below it.[34] C5 bears homology with C3 and C4, but lacks an internal thioester residue. Cleavage of C5 by C5 convertases results in release of the C5a fragment, a potent anaphylatoxin and chemoattractant.

Membrane attack complex is subsequently formed by the sequential fusion of C6, C7, C8, and C9 to C5b. Fusion of the molecules is accompanied by hydrophilic-amphiphilic transition of the complex and results in the generation of an integral membrane attack complex. C7 plays a critical role in the hydrophilic–amphiphilic transition because it confers on the intermediate complex C5b-7 the transient ability to bind directly to target cell membranes.[35,36] The membrane-bound C5b-7 complex facilitates the incorporation of a C8 molecule and subsequently several C9 molecules into the membrane attack complex resulting in formation of a poly-C9 transmembrane pore.

The site (or "quality") of C5b-9 insertion may be an important factor in determining whether *Neisseria* are killed by complement. As an example, an anti-meningococcal porin A (PorA) antibody

enhanced killing of bacteria in a dose-related manner, but did not increase the total amount of C5b-9 on the organism.[37] Similarly, a comparison of serum-sensitive and serum resistant *Ng* revealed that both organisms bound similar amounts of C7 and C9,[38] but the C5b-9 complexes associated with distinct proteins on resistant vs. sensitive strains.[39]

Regulation of Complement Activation

The labile nature of complement necessitates its strict control under normal physiological conditions to prevent needless damage to host tissues. Several fluid-phase and soluble molecules effect complement regulation (summarized in **Table 2**). The molecules that have been studied in the context of meningococcal pathogenesis thus far are discussed in more detail below.

C4b-binding protein

C4b-binding protein (C4BP) is a key soluble phase inhibitory protein of the CP and functions as a cofactor for the factor I-mediated cleavage of C4b (cofactor activity) and also dissociates C2a from the CP C3 convertase, C4b,2a (decay acceleration). C4BP is a "spider-like" molecule with 6 or 7 elongated tentacles (α-chains) extending from a central, ring-like core.[40,41] Each α-chain comprises 8 short consensus repeat (SCR) domains. Each SCR domain contains four highly conserved Cys residues and several conserved amino acids. Some isoforms of C4BP also contain a β-chain that comprises three SCRs. The major C4BP isoform is comprised of seven α-chains and one β-chain (α7β1), while α7β0 and α6β1 are the two other described isoforms. The β-chain of C4BP is in a complex with protein S of the activated protein C pathway. Protein S functions as a cofactor to activated protein C in the degradation of coagulation factors Va and VIIIa. Only free protein S, but not protein S bound the β-chain of C4BP, functions in the protein C anti-coagulation pathway. This constitutes one of several links between the complement, coagulation and fibrinolytic pathways and the reader is referred to Oikonomopoulou et al. for an excellent in-depth review on this topic.[42]

Factor H and the factor H family of proteins

Factor H (fH) is the key regulator of the AP of complement. fH acts as a cofactor for the factor I mediated cleavage of C3b to iC3b (cofactor activity) and also serves to irreversibly dissociate Bb from the AP C3 convertase, C3b,Bb, (decay-accelerating activity). fH is a 150-kD single-chain glycoprotein composed entirely of 20 SCR domains,[43] each comprising 60 amino acid residues. The 20 SCR domains are arranged in the form of a "string-of-beads" connected by 19 short, potentially flexible linkers[44-46] of 3 to 8 amino acids each. The N-terminal 4 SCR domains of fH are necessary for cofactor and decay accelerating activity. The plasma concentrations of fH range from 200 to 600 μg/ml.[47-50] Although the liver is the main site of fH synthesis, fH is secreted by a variety of cells including retinal pigment epithelial cells, proximal renal tubular epithelial cells and endothelial cells.

An alternatively spliced variant of fH called fH-like protein 1 (FHL-1), contains the seven N-terminal SCRs of fH with the exception that SCR7 of FHL-1 is spliced to a separate exon that encodes four unique C-terminal amino acids (SFTL).[51] FHL-1 possesses cofactor and decay accelerating activity because it contains the complement regulating regions of fH (SCRs 1 through 4).

A family of SCR-containing proteins that bear varying degrees of homology to fH, called fH-related proteins (FHRs), have been described. The genes coding for these proteins, in the order *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, and *CFHR5*, are located 3′ to *CFH* (the gene encoding fH).[52] The FHRs are believed to modulate complement regulation by fH through their interactions with surface-bound C3 fragments.[53]

Vitronectin

Vitronectin (also called S-protein or serum spreading factor) is an important extracellular matrix molecule and inhibitor of the terminal complex that circulates in plasma (200–400 μg/ml) as a folded monomer of a single chain (75 kDa) or a cleaved structure containing two chains (10 and 65 kDa) held together by a disulfide bond.[54] Vitronectin is conformationally labile and formation of complexes with plasminogen activator inhibitor-1 (PAI-1),[55,56] thrombin-antithrombin III[57] or C5b-9 induces conformational changes in vitronectin that expose a heparin binding domain, expose the N-terminal somatomedin B (SMB) domain and lead to formation of disulfide linked multimers of vitronectin.[58] The SMB domain contains an Arg–Gly–Asp (RGD) sequence that binds to integrins ($\alpha_V\beta_3$, $\alpha_V\beta_5$, $\alpha_V\beta_1$, and $\alpha_{IIb}\beta_3$) and serves as a cell attachment site.

Vitronectin can inhibit the terminal complement complex at various stages. The earliest structure in the formation of the membrane attack complex that can insert into a membrane is C5b-7 and vitronectin can occupy the metastable membrane-binding site of the nascent C5b-7 complex[59] to form SC5b-7. The SC5b-7 complex can take up further C8 and C9 molecules to form SC5b-8 and SC5b-9, respectively,[60] but these latter complexes lack hemolytic activity. The wedge-shaped ultrastructure of vitronectin-containing membrane attack complexes differs from the circular complement lesions that mediate hemolytic activity.[61] Vitronectin can also block C9 polymerization[62] and limits the number of C9 molecules in the membrane attack complex to three. Killing of *E. coli* requires more than three C9 molecules per C5b-9 complex,[63] thus limiting C9 polymerization by vitronectin may serve as a complement evasion strategy. Vitronectin is a multifunctional protein and in addition to complement regulation, vitronectin also blocks pore formation by perforin[64] and plays roles in coagulation, fibrinolysis, pericellular proteolysis, vascular remodeling, cell attachment, and spreading.[65,66]

Membrane-bound complement regulators

Several cell membrane-associated proteins serve to limit unwanted complement activation on host tissues, thereby limiting unnecessary complement activation and "bystander" host cell damage when complement is being activated on invading microbes. **Table 2** lists these cell-associated regulators and their functions. Molecules relevant to meningococcal pathogenesis are discussed below.

Membrane cofactor protein (MCP; CD46)

CD46 is also known as membrane cofactor protein (MCP) because it serves as a cofactor in the factor I-mediated cleavage of

C3b and C4b to iC3b and C4d, respectively. It is expressed on most nucleated cells and comprises four SCR domains followed by an O-glycosylated serine/threonine/proline-rich (STP) domain, 12 residues of unknown function and an intracellular domain. CD46 is a type 1 membrane glycoprotein and alternative splicing results in its expression as four common isoforms called C1, C2, BC1, and BC2. N-glycans of SCR 2 and 4 are necessary for cofactor activity.[67] Glycosylation of the STP domain modulates its functional activity; isoforms with the larger O-glycosylation domain (BC isoforms) bind C4b more efficiently and provide enhanced cytoprotection against the CP than the smaller, less glycosylated, C isoforms.[68]

Complement receptor 1

Complement receptor 1 (CR1), also called CD35, or the immune adherence receptor, is an integral membrane glycoprotein that is composed of a C-terminal transmembrane region and an extracellular linear array of 30 SCR units.[69,70] The N-terminal 28 SCRs are organized as four tandem, long homologous repeats (LHRs) of seven SCR units each.[69,70] CR1 is a receptor for C3b, C4b, C1q, and MBL. Human erythrocyte CR1 mediates binding of complement-opsonized immune complexes or microorganisms to the cell and this forms the basis for the phenomenon of immune adherence.[71] These bound complexes or organisms are carried to the spleen or liver where they are removed; in the process the CR1 molecule is also lost from the RBC surface.[72-76] Studies in vitro using whole human blood showed that binding of complement-coated meningococci to CR1 on RBCs delayed their uptake by phagocytes.[77] However, a role for CR1 in meningococcal disease has not yet been elucidated.

Correlates of Protection against Meningococcal Disease

Complement-mediated bactericidal antibodies

Role of antibodies and complement in protection

Flexner and Jobling demonstrated in the early 1900s that subdural (intrathecal) administration of equine anti-meningococcal antiserum reduced the mortality of meningococcal meningitis and suggested a role for antibodies in protection against meningococcal meningitis.[78,79] According to Flexner's report, a pandemic of meningococcal disease that began in 1904 resulted in a mortality rate in untreated individuals of about 80%. Intrathecal serotherapy was performed in about 1300 patients and reduced the overall mortality to 31%. Flexner also made the astute observation that antibody therapy was associated with greater engulfment of bacteria by leukocytes.

The seminal studies of Goldschneider and colleagues published in 1969 demonstrated the importance of complement-dependent killing by antibody in protection against meningococcal disease.[80] They initially studied the ability of sera obtained from 282 children and 567 army recruits and observed an inverse relationship between the incidence of meningococcal disease and age-specific serum bactericidal activity against prototypic group A, B and C meningococcal strains. These experiments were performed with an exogenous human complement source. The authors then conducted a prospective study on new recruits at the US Army base at Fort Dix, New Jersey.[81] Baseline serum samples were collected over four months from 14744 recruits during their first week of basic training and stored at −70 °C to preserve endogenous complement activity in the sera. During this 4 month period, 60 cases of invasive group C meningococcal infection occurred, which represented an attack rate of 0.5 per 100 recruits over a 4 week period, about 300 times above that seen in the general population. The homologous meningococcal isolate that caused disease was available in 54 individuals from whom baseline sera had been obtained. Only 5.6% of these baseline sera had bactericidal activity against the homologous strain. By contrast, about 82% of baseline sera from recruits who did not get meningococcal disease showed bactericidal activity against the meningococcal disease isolate. A more recent study of an outbreak of group C disease in 6 students at a university in the UK[82] supports the earlier observation of Goldschneider et al. Analysis of acute sera from three individuals that died revealed that none exhibited bactericidal activity against the infecting strain. These data support the proposal that acquiring a virulent group C isolate in the absence of protective bactericidal titers is more likely to lead to invasive disease.

The importance of complement-mediated bactericidal activity in protection against meningococcal disease is also underscored by the high incidence of invasive meningococcal disease in persons with terminal complement deficiencies (C5 through C9),[31,83] whose sera can support opsonophagocytosis through engagement of Fcγ-receptors and/or receptors for C3 fragments on professional phagocytes but whose sera cannot support direct complement-mediated lysis. Normal opsonic function in late complement component-deficient (LCCD) individuals is suggested by the lack of increased incidence of pneumococcal infections in this population. The requirement for protection through membrane attack complex appears to be unique for *Nm* and for disseminated gonococcal infections; terminal complement deficient patients are not at a greater risk for contracting other gram-negative infections.

Evidence for non-complement-mediated killing mechanisms

Not all persons in the Goldschneider study who acquired the epidemic isolate in their nasopharynx and were considered susceptible to disease by SBA criterion developed invasive disease.[81] Baseline serum (drawn within a week of recruitment) and serial nasopharyngeal cultures (taken during weeks 1, 3, 5, and 7 of basic training) were obtained from 492 men from three separate companies at Fort Dix. Five cases of meningococcal disease occurred among these men during the study period. Of the 492 men, 54 (11%) had baseline serum bactericidal titers less than 1:4 against the epidemic circulating strain and were considered susceptible. Twenty-four of the 54 susceptible recruits became colonized with the group C epidemic isolate; 11 of these 24 men developed serum bactericidal titers. Of the remaining 13 men who were colonized with the disease causing isolate and considered susceptible by serum bactericidal criteria, only 5 developed invasive disease, yielding an attack rate of 38.5%. These data suggest a possible role for mechanisms other than complement-mediated bactericidal activity in protection against invasive meningococcal disease. Similar observations

were made by Trotter et al., who studied the correlation between the prevalence of group B disease and serum bactericidal titers in >2400 individuals in the UK.[84] As shown in several prior studies, the highest incidence of disease was seen in infants under 1 y of age. The peak incidence occurred between 3 and 7 mo of age, which also coincided with low SBA titers. While the disease incidence declined rapidly after 1 y of age, this was not accompanied by a corresponding increase in the proportion of individuals with SBA titers >1:4. Thus, factors other than direct complement-mediated lysis may contribute to protection against invasive disease. Antecedent viral (e.g., influenza A), mycoplasma respiratory infections,[85] and exposure to cigarette smoke[86,87] are all associated with an increased risk of invasive meningococcal infection. These factors presumably increase expression of adhesion molecules for meningococci in the nasopharynx and/or alter the local cytokine milieu to facilitate bacterial invasion of the bloodstream.[88,89] A possible role for opsonophagocytosis in protection is discussed below.

Opsonophagocytosis in protection against meningococcal infections

Resistance to complement-dependent lysis and opsonophagocytosis is not mutually exclusive—as an example, limiting antibody binding decreases C3 fragment deposition, which in turn will reduce both opsonophagocytosis and complement-mediated lysis.

Role of the meningococcal capsule in resistance to opsonophagocytosis

Meningococcal capsular polysaccharide is a determinant of complement resistance (unencapsulated isolates or mutants are sensitive to killing by normal human serum) however it is also anti-phagocytic and assists the bacterium in overcoming this arm of immune defense. The importance of capsular polysaccharide in combating opsonophagocytosis is best realized in individuals with terminal complement defects. If the only function of the polysaccharide capsule was to prevent direct killing by complement during invasive infection, then LCCD patients, whose serum is incapable of forming the MAC, should be predisposed to invasive infections by unencapsulated *Nm* isolates, such as those that often colonize the nasopharynx. However, meningococci isolated from the blood or cerebrospinal fluid of LCCD individuals are almost always encapsulated and resistant to killing by normal human serum that contains intact complement.[31] Opsonophagocytosis likely prevents unencapsulated bacteria from invading the bloodstream and causing disease.

Some strains of *Nm* may be relatively more resistant to opsonophagocytosis than others[90] and it is possible that such strains may preferentially cause disease in terminal complement deficient persons, whose sera cannot directly kill bacteria but can coat organisms with C3 fragments to facilitate phagocytosis. Some reports suggest that individuals with terminal complement defects appear to have a higher incidence of disease caused by groups W, Y and other rare groups compared with normal individuals,[91,92] although one study showed no differences between isolates from complement-deficient and -sufficient persons.[93] Small, retrospective studies have shown that the incidence of meningococcal disease in LCCD individuals decreased following immunization with polysaccharide vaccines, supporting a role for opsonophagocytosis in protection against disease.[94]

Taken together these studies demonstrate that, although antibody-mediated complement-dependent bactericidal killing is a well-documented correlate of protection against meningococcal disease, opsonophagocytosis also plays a discernible role.

Genetic polymorphisms and meningococcal disease

Fcγ receptors

Fcγ receptors found on phagocytic cells bind to the Fc portion of IgG molecules attached to microorganisms and facilitate phagocytosis; antibodies are particularly important for enhancing phagocytosis of encapsulated bacteria. Associations between meningococcal disease and genetic variants of Fcγ that impair function support a role for opsonophagocytosis in preventing infection. The Fcγ receptor family in humans is broadly divided into FcγRI, FcγRII, and FcγRIII; FcγII is further subdivided into FcγRIIA (CD32), FcγRIIB, and FcγRIIC and FcγRIII is subdivided into FcγRIIIA and FcγRIIIB (CD16).[95] A variety of human FcγR alleles with altered functionality exist; the role of two Fcγ alleles in *Nm* that have been investigated are the H/R131 forms of FcγRIIA and the NA1/NA2 forms of FcγRIIIB. The H/R alleles at amino acid 131 of FcγRIIA determine the affinity for human IgG2, which is the dominant IgG subclass response to encapsulated pathogens.[96] H/H homozygosity results in high affinity binding, R/R homozygosity results in low affinity binding, while heterozygotes show intermediate affinity for IgG Fc. Studies in vitro have demonstrated that IgG1-mediated phagocytosis of *S. aureus*, *H. influenzae* type b, and group B meningococci was impaired in PMNs homozygous for the NA1 allele of FcγRIIIB compared with PMNs with the NA2 allele.[97] The differences were most apparent in the absence of complement and with concomitant blockade of FcγRIIA.

In a study of 15 persons from four families with C6 or C8 deficiency either with (n = 8) or without (n = 7) a history of meningococcal disease, the combined FcγRIIA (R/R 131)/FcγRIIIB (NA2/NA2) phenotype was seen in 6 individuals with a history of disease and in only one without a history of meningococcal disease (odds ratio, 13.9). The authors also examined 15 properdin-deficient individuals from 5 families; 7 individuals experienced a single episode of meningococcal disease and 8 were disease-free. There was no association noted between FcγRIIA phenotype and disease susceptibility in this properdin-deficient population. In a retrospective study of 25 children who survived fulminant meningococcal septic shock, 11 were homozygous for the R/R131 allele of FcγRIIA (binds IgG2 with lower affinity than the H/H allele).[98] The incidence of the R/R allele in these sepsis survivors was higher than the prevalence of the same allele in control healthy Caucasians (44% vs. 23%). PMNs from persons with the R/R131 allotype phagocytosed *Nm* opsonized with polyclonal IgG2 antibodies less effectively than PMNs derived from H/H131 individuals.

FcγRIIA allotypes were evaluated in 29 Russian terminal complement-deficient patients (28 were C8-deficient and one

patient had C7 deficiency) who suffered one or more episodes of meningococcal disease.[99] A total of 63 episodes of meningococcal disease were experienced by these 29 unimmunized individuals between 1961 and 1994; 9 patients experienced a single episode, 10 had two, 4 had three, 3 had four, and two had five episodes of disease. The risk of contracting meningococcal disease was 3.3 times higher in R/R131 homozygous individuals above the age of 10 than in the corresponding age-matched H/H homozygous individuals. H/H homozygosity was not protective in children under 10 y of age. Age-related protection of the H/H allele may be the result of higher acquired bacteria-specific antibody levels in older individuals than in the younger population and consequently, protection mediated by FcR would be best revealed in the presence of antibody. In the same study, meningococcal disease classified as "severe" was also more frequent in patients with R/R and R/H allotypes, compared with patients with the H/H131 allotype. Thus, H/H131 allotype may facilitate antibody-mediated phagocytosis of meningococci in patients above 10 y of age, which could be particularly important in the face of LCCD. More efficient phagocytosis may limit bacterial burden and result in a relatively milder disease course in H/H homozygotes.

Factor H and factor H-related proteins

Binding of the alternative complement inhibitor, fH, to meningococci enhances their ability to evade killing by complement (discussed below). A genome-wide association study (GWAS) to define host susceptibility to meningococcal disease was performed using 475 individuals with invasive meningococcal disease and 4703 controls in the UK.[100] Two additional replication studies were performed in western European and southern European individuals (968 cases and 1376 controls) to validate the most significant SNPs. In both studies, a SNP within complement fH (CFH) (rs1065489 [p.936D < E], $P = 2.2 \times 10^{-11}$) and in CFH-related protein 3 (CFHR3) (rs426736, $P = 4.6 \times 10^{-13}$) replicated independently. A previous smaller study had linked meningococcal disease with the C/C homozygous genotype in the NFκB responsive element at position −496 that is associated with higher serum fH levels.[101] However, this association was not observed in the study by Davila et al.[100]

Complement Activation and Disease Severity in Meningococcal Disease

Nm is most feared for its propensity to cause a rapidly evolving meningitis and life threatening sepsis. Disease severity is directly related to bacterial burden, which typically ranges from approximately 10^4 to 10^8 cfu/ml of blood; loads as high as 10^9 cfu/ml have been measured during fulminant meningococcemia. A study of 1045 patients throughout England and Wales showed, using real-time quantitative PCR to estimate the number of bacteria, that higher bacterial loads correlated with poor outcomes.[102] Higher estimated bacterial loads were associated with prolonged hospitalization, digit, limb or soft tissue loss and the need for hemodialysis. This study corroborated results from a previous study which also showed a correlation between higher bacterial load in severe disease (median 8.4×10^6 DNA copies/ml) compared with milder disease (1.1×10^6 DNA copies/ml).[103] Bacterial load did not correlate with the duration of symptoms prior to admission.

Role of LOS, inflammatory mediators, and complement in disease severity

LOS and inflammation

Endotoxin (LPS or LOS) is the bacterial molecule responsible for stimulating the release of inflammatory mediators and disease severity is also directly correlated with the level of endotoxin and circulating levels of proinflammatory cytokines (TNF-α, IL-1, and IL-6)[104,105] and chemokines (monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein [MIP]-1 α, and IL-8).[106] High levels of circulating endotoxin during meningococcal infection result from the large numbers of bacteria in the bloodstream as well as from outer membrane fragments called "blebs" that are shed by the bacteria. Hellerud et al. used a porcine model to elucidate the roles of LOS and non-LOS components of *Nm* in vivo. Norwegian landrace pigs were inoculated intravenously with increasing doses (ranging from 5.7×10^{10} to ~10^{12}) of wild-type strain H44/76 and its LOS-deficient mutant. Several cardiopulmonary, hematologic and inflammatory parameters were monitored. The results showed that LOS was largely responsible for inciting inflammation and for inducing a "sepsis-like" physiology. It took 10- to 20-fold higher inocula of the LOS-deficient mutant to induce a similar degree of pathophysiology as the wild-type isolate. In addition, other studies have shown that an LOS-deficient meningococcus is significantly less potent in inducing chemokines and cytokines (IL-1β, TNF-α, IL-8, and MIP-1α) than wild-type bacteria. The specific structure of the lipid A molecule is important in inciting inflammation. Fransen et al.[107] screened 464 meningococcal isolates for their ability to stimulate cytokine release from monocytes/macrophage cell lines in vitro and found that 9% elicited a weak cytokine response. These isolates expressed penta-acylated lipid A (*Nm* LOS is usually hexa-acylated) because of mutations in the *lpxL1* gene. Patients infected with these *lpxL1* mutant strains presented significantly less frequently with rash, had higher platelet counts, and less activation of tissue-factor mediated coagulopathy. Ladhani and colleagues characterized 114 group Y strains isolated between 2007 and 2009 in England and Wales.[108] Sixty-four (56%) group Y strains belonged to clonal complex (cc) 23 and 62 of these 64 (97%) cc23 strains harbored *lpxL1* mutations that resulted in loss of LpxL1 expression. The cc23 strains affected younger (<25 y) patients and caused meningitis more often, but were associated with similar mortality rates when compared with non-cc23 isolates. These data point to an important role for *Nm* lipid A structure in determining the clinical course of meningococcal disease.

LOS and complement

Brandtzaeg, Mollnes, and coworkers correlated the severity of disease manifestation with circulating endotoxin levels (measured by the Limulus amoebocyte lysate assay) and complement activation products (C3 activation and C5b-9 levels).[109] The spectrum of disease seen among 39 individuals included fulminant meningococcemia (severe septic shock), meningococcemia (no evidence of septic shock or meningitis),

and meningitis. Fulminant disease was associated with higher levels of LOS and higher concentrations of complement activation products in serum when compared with the two other groups. Further, 7 of the 13 individuals with fulminant disease who survived had significantly higher C5b-9 levels than the 6 who succumbed to the infection. A direct correlation between LOS and complement activation was noted. Peak complement activation in the surviving patients occurred between 0 and 44 h (median 7 h) following admission and declined rapidly thereafter; 6 d after admission, levels were no longer significantly higher than baseline (6 week) levels.

The interrelationship between complement activation, endotoxin levels and disease severity is complex. Complement activation by *Nm* appears to be independent of LOS expression as evidenced by observations that purified LOS is a poor activator of complement and that an LOS-deficient meningococcal mutant activated complement to the similar extent as the wild-type strains that expressed LOS.[110] Studies in vitro have shown that complement activation by meningococci incubated with serum occurs when bacteria are present at concentrations >10^6 CFU/ml;[110] uncontrolled complement activation (measured by soluble C5b-9 generation) is seen only above >10^7 bacteria/ml.[111] The relationship between disease severity and complement activation may involve the ability of activated complement to bring about the release of endotoxin (LOS) which in turn exacerbates disease progression. Tesh and colleagues showed that *E. coli* strain J5 released LPS into the supernatant when incubated with normal human serum.[112] LPS release was abrogated when serum was inactivated by heating to 56 °C for 30 min, which destroys complement activity. O'Hara et al. showed that LPS release from *E. coli* J5 did not occur in C9-deficient serum, but was restored when the serum was reconstituted with pure C9.[113] Collectively, these studies suggest that LPS release—at least in the instance of *E. coli*—required an intact complement system. The latter scenario may serve to explain why persons with terminal complement deficiencies have a more favorable diseases outcome and is discussed below. The ability of activated complement to release endotoxin and enhance disease severity is of particular interest and relevant to the clinical course of invasive disease in terminal complement deficient individuals, as discussed further below.

Complement Deficiency and Susceptibility to Meningococcal Disease

An apparent strong association between complement deficiencies and invasive neisserial infections prompted several studies to estimate the frequency of complement deficiencies among patients with their first episode of systemic neisserial infection.[91,114-126] Figueroa and Densen[31] studied the correlation between the incidence of complement deficiency in patients with meningococcal disease vs. the incidence of meningococcal disease in the general population and showed that the rate of complement deficiencies detected dropped in areas the incidence of disease was high. For example, 0 of 47 individuals with meningococcal disease in Denmark, where the incidence was ~3.5 cases per 1 000 000 population, had complement deficiencies.[125] In contrast, complement deficiency was diagnosed in 8 of 16 persons (50%) with meningococcal disease in Japan, where the incidence of disease was comparatively lower (0.1 in 1 000 000).[122] These data indicate that complement deficiencies may be less of a contributing factor as the incidence of disease increases, for example during epidemics. The introduction of a hypervirulent clone into a non-immune population would result in disease and spread of the strain efficiently among normal as well as complement deficient individuals; because the number of the normal individuals greatly exceeds the number of complement-deficient individuals, the relative proportion of complement deficient persons affected will be small. In non-epidemic situations, or where the population at large has developed immunity against the prevalent strain, the complement-deficient individuals are likely to be at a higher risk and would represent a greater proportion of cases.

Complement deficiency states may be either acquired or inherited. Inherited deficiencies of complement are rare and occur in about 0.03% of the general population. The incidence varies widely depending on the complement protein and the ethnicity of the population studied. While hereditary deficiency of C2 is relatively common (~0.01%), complete deficiencies of other proteins such as C4 are very rare. C9 deficiency is relatively common in the Japanese population, and is seen in ~0.1% of blood donors. This contrasts with the low frequency (0.005%) of C7 deficiency in the same population. C7 deficiency, however, is relatively common in the Israeli Moroccan Jewish population; of a total of 365 healthy blood donors, one was homozygous (0.27%) and six were heterozygous (1.6%) for the mutant allele.[127] A relatively high frequency of C6 deficiency has been detected in the Western Cape population of South Africa[128,129] and among African-Americans (approximately 1 in 1600) in the southeastern United States.[130]

Acquired deficiencies are more common and can result from inadequate production of complement components (e.g., severe liver dysfunction), increased consumption of complement (autoimmune disorders, diseases associated with immune complex formation), or increased excretion of complement components (e.g., protein-losing nephropathies). Conditions associated with impaired antibody production, such as splenectomy, may also be a predisposing factor for meningococcal disease.[131-133] *Streptococcus pneumoniae* is overwhelmingly the most common infectious complication in splenectomized persons, who are at a several hundred-fold increased risk of invasive disease compared with normal individuals. The relative increase in risk of meningococcal infections in splenectomized persons is not known, but because bacterial infections in these individuals often follows a fulminant course with high mortality,[131,134] it has led to the recommendation to immunize this population against pneumococcal, meningococcal, and *Haemophilus influenzae* type b infections.[135,136]

Inherited deficiencies of complement and meningococcal infections

Inherited deficiencies of C3, the AP (factor D, properdin, fH, and factor I) and terminal complement pathway components (C5 through C9) are all strongly associated with an increased

incidence of invasive meningococcal infections. CP defects are more associated with autoimmune diseases[137] and the relatively low incidence (~20%) of infections in persons with CP deficiencies has been attributed to an intact AP. Bacterial infections in persons with CP deficiencies are usually caused by encapsulated bacteria such as *Streptococcus pneumoniae*,[31,32] may be recurrent and often involve the sino-pulmonary tract, meninges, and blood. Deficiencies in the lectin pathway, specifically decreased levels of MBL, may also be associated with increased meningococcal disease and this is discussed below.

Deficiencies of C3 and the AP

The AP is important in combating infection with *Nm* and although AP deficiencies are relatively rare, they are associated with increased incidence of meningococcal disease. Primary C3 deficiency is rare, with only about 20 cases reported in the literature. Because of its central position in the complement cascade and the variety of functions it serves, which include neutrophil chemotaxis, opsonophagocytosis, and serum bactericidal activity, these individual suffer severe, recurrent infections caused by *S. pneumoniae*, *Haemophilus influenzae*, and *Nm*.[31,32] These infections usually involve the respiratory tract, bloodstream and the meninges. fH and factor I deficiencies result in uncontrolled activation of the AP and consumption of complement, thereby creating a functionally complement deficient state that predisposes these individuals to meningococcal disease.[31,32]

Factor D deficiency is very rare. Hiemstra et al. described a 24-y-old man who was diagnosed with disseminated gonococcal infection (DGI; blood cultures were positive for *N. gonorrhoeae*) and was diagnosed with complete factor D deficiency. This individual had a prior episode of DGI (also with positive blood cultures) at 19 y of age and an episode of meningococcal meningitis at the age of 14. Subsequently, Biesma and colleagues described a family with a high degree of consanguinity where five members were diagnosed with complete factor D deficiency.[138] The index case was a 23-y-old woman with group B meningococcemia. An older family member with complete factor D deficiency had a history of meningitis during military service in 1949. Of note, the index case's twin sister, mother, and maternal uncle all were completely factor D-deficient but did not suffer from infections. Sprong et al. described group B meningococcemia secondary to factor D deficiency in a 13-mo-old child of consanguineous parents of Turkish descent.[139] The sibling of the index case had previously suffered a fatal episode of group B meningococcemia at 9 mo of age; genetic studies on the deceased child were not performed.

Approximately 70 cases of properdin (stabilizes the alternative pathway C3/C5 convertases) deficiency have been reported.[31] Defects of factor D and properdin are associated with an increased predisposition to meningococcal disease, which brings these deficiencies to clinical attention. Disease in these individuals may be severe,[140,141] in contrast to the milder disease observed in late complement component deficient individuals as discussed below. In AP-deficient persons with high titers of specific antibody, the CP can be activated normally and may mediate bactericidal activity. However, in the absence of specific antibody, C3 fragment deposition on and killing of meningococci is markedly impaired and renders such persons highly susceptible to invasive disease. AP defects do not appear to be associated with a higher incidence of infections caused by pathogens other than *Nm*.

Late complement component deficiencies and meningococcal disease

Terminal complement deficiency is associated with a 7000- to 10 000-fold higher risk of developing meningococcal disease.[31] The risk of disease in C9-deficient individuals is comparatively lower (~1400-fold increased risk compared to normal individuals) as discussed above, probably because C9-deficient serum can support hemolytic activity, albeit very weakly.[31] About 40–50% of terminal complement deficient persons suffer recurrent infections. In some instances, relapses have been documented, defined as infection recurring with the same group within one month of the prior infection. The observation that terminal complement deficient persons get repeated infections raises the possibility that each bout of infection does not provide protection against subsequent episodes of disease. Platonov et al. examined 77 episodes of meningococcal disease in 30 individuals with LCCD (27 with C8 deficiency and 3 with C7 deficiency).[124] Males predominated among the 24 individuals who had more than one episode of invasive disease (21 males to 3 females). The median age of the LCCD persons at the time of their first episode of meningococcal disease was 15 y (range 1–46). Using mathematical modeling, the authors showed that the probability of LCCD individuals developing a subsequent episode of meningococcal infection was independent of having had a previous episode(s)—that is, prior meningococcal disease did not protect these patients from subsequent episodes. The risk of acquiring each episode of infection was about 56%. Figueroa and Densen applied a similar calculation on a large number of cases compiled from the literature and arrived at similar conclusions, with a calculated risk of each infection of 39.1%.[31] The reason for a lack of protection of LCCD patients against subsequent attacks of disease has not been fully elucidated. These individuals mount an antibody response against the infecting isolate that in most instances can support killing of the homologous isolate when complement sufficient serum is used as a complement source.[83] The opsonophagocytic activity of these antibodies that the LCCD individuals rely on for protection against disease may have limited activity against heterologous isolates. Further, the antigenic specificity of the elicited antibodies has not been fully characterized. The relative concentrations of protective antibodies and subversive "blocking" antibodies (discussed below) over time following infections may also be a factor that determines why some but not all individuals with LCCD suffer recurrent infections.

Although predisposed to recurrent episodes of invasive disease, individuals with LCCD enjoy a lower mortality per episode of meningococcal disease than normal hosts.[124] By contrast, meningococcal disease in persons with AP defects (properdin and factor D deficiencies) does not follow a milder clinical course.[124] A factor that may contribute to the milder disease course in these individuals is lower endotoxin release from the bacterial surface in the absence of an intact terminal complement pathway as discussed above. The correlation between endotoxin

release and an intact complement system was illustrated in a case reported by Lehner et al.[142] A 29-y-old woman was admitted with meningococcal septicemia and meningitis. She was treated with penicillin and was administered 2 units of fresh frozen plasma (FFP) in an attempt to correct ongoing coagulopathy. She also received an experimental anti-TNF antibody. The patient's mental status worsened over the first 8 h, but she subsequently recovered. Group Y *Nm* was isolated from the blood and the cerebrospinal fluid. It was subsequently determined that the patient was C6-deficient. An analysis of serial serum samples obtained during the patient's hospitalization revealed that FFP administration transiently restored C6 concentrations, which was associated temporally with an abrupt increase in serum LPS concentrations. Further analyses showed that serum samples taken from this patient shortly after admission did not cause release of LPS from *E. coli* J5, but samples obtained following infusion of fresh frozen plasma did release endotoxin from *E. coli*. These data provided evidence for lower endotoxin levels in a human with a terminal complement defect and meningococcemia and also urges caution while administering blood products such as fresh frozen plasma during meningococcal sepsis that can restore hemolytic activity in LCCD patients.

MBL and meningococcal disease

Case reports and studies of individual families have suggested an association between low MBL levels and meningococcal disease.[143-145] The combination of MBL and properdin deficiency may heighten the predisposition to meningococcal disease. In a Danish family where 4 members suffered meningococcal disease (with one fatality), 2 of 6 males with undetectable properdin levels had meningitis and both these patients had MBL variant alleles that predicted low MBL levels. High MBL concentrations were seen in 3 of the remaining 4 properdin-deficient males and none had meningitis.[146]

Population-based studies further substantiate a role for MBL in defenses against meningococcal disease. The frequency of variants of the MBL gene was ascertained in children in the United Kingdom with meningococcal disease and controls from two independent studies. One study was hospital-based (194 patients and 272 controls patients with non-infectious disorders), and the other was community-based (72 patients and 110 control healthy individuals). The proportion of people homozygous for MBL-variant alleles was higher in patients with meningococcal disease than in controls in both the hospital study (7.7% vs. 1.5%) and in the community study (8.3% vs. 2.7%).[147] It should be noted that the frequency of homozygosity for MBL variant alleles in the control groups in this study were far lower than that reported for people of European ancestry (about 23%). Further, this study did not adequately match cases and controls for ancestry. In a previous study in Norwegian patients with meningococcal disease (76 with group B and 25 with group C disease), the proportion of individuals with low MBL levels (defined as less than 100 ng/ml) was similar in cases vs. healthy blood donor controls (10.1% vs. 12.5%).[148] A key difference in the two studies was that the mean age in the UK hospital cohort was 3.5 y, compared with 16 y in the Norwegian group. More recently, mutations in exon 1 of *mbl2* that determine low MBL levels (codons 54, 52, and 57) were examined in a pediatric cohort (ages 2–215 mo) with invasive meningococcal disease and compared with healthy age-matched volunteers and no history of meningococcal disease.[149] The overall frequency of a MBL exon 1 variant genotype was significantly higher in patients than in controls (31.8% vs. 8.2%). When stratified according to age, 39.3% of patients with disease onset less than 24 mo of age had a MBL structural variant genotype. This was further increased and most pronounced in children with disease onset less than 12 mo of age (57.1%). Clinical severity and outcome did not differ between patients with wild-type and mutant alleles.[149] Again, the low frequency of MBL variant alleles reported in the control population in this study should be noted. In the largest case-control study to date, Bradley et al. compared 296 cases with 5196 controls, all of European ancestry, and did not demonstrate an association between MBL variant alleles and meningococcal disease overall, or in any age-defined subgroup.[150] While the role of MBL in protection against meningococcal disease remains unclear, the lectin pathway may possibly play a role in protection against meningococcal disease in early childhood prior to maturation of the adaptive immune system.[151] It should be noted that studies that rely solely on MBL haplotypes as a correlate (or surrogate) of plasma MBL levels need to be interpreted with caution because MBL levels vary considerably across individuals with identical haplotypes, or even in the same individual following acute infection or major surgery.[152]

Acquired deficiencies of complement and meningococcal infections

While it is well established that inherited deficiencies of complement constitute a strong risk factor for meningococcal disease, the risk of meningococcal disease in persons with acquired complement defects is less clear. Some studies have noted associations between acquired complement deficiencies and meningococcal disease and it seems logical that these deficiencies put an individual at increased risk for infection, however, extensive studies with large numbers of cases are currently lacking. Acquired complement deficiencies are more common than inherited ones and often result in multiple complement defects at once. For example, the complement profiles of two patients with severe hepatic failure that was complicated with meningococcemia were studied by Ellison et al.,[153] who documented normal C1q levels, but low or undetectable concentrations of C3 through C6, C8, C9, factor B, and factor I.

Immune complexes associated with autoimmune disorders activate and consume complement; ~50% of patients with systemic lupus erythematosus (SLE) are deficient in C3 and C4. These conditions may also predispose individuals to meningococcal disease. Of 20 patients presenting with their first episode of invasive meningococcal disease, 6 had a CH50 (also called "total hemolytic activity") level less than 2 standard deviations below the normal mean.[118] Of these patients, three had inherited complement deficiencies (C6 or C8) and the remaining three had acquired defects that were associated with either SLE or multiple myeloma. Further, Garty et al.[154] found that 3 of 30 patients with invasive meningococcal disease in Israel between 1970 and 1989 had complement deficiencies; one patient had

inherited C7 deficiency, while the other two patients had SLE and membranoproliferative glomerulonephritis (MPGN). The latter two patients had low C3 and C4 and CH50 levels prior to the onset of infection. The incidence of meningococcal infection in the Jewish population of central Israel was estimated to be 1/100 000 and that of SLE with MPGN ~250/100 000. The concomitant occurrence of these two rare entities supported an association between acquired complement deficiencies and meningococcal disease. Reports by Feliciano et al.[155] and Mitchell et al.[156] further supported an association between SLE and meningococcal disease.

C3 nephritic factor (C3 NeF) is an autoantibody that stabilizes the AP C3 convertase (C3b,Bb) and results in C3 activation and consumption. Further, uncontrolled complement activation results in complement-containing deposits within the glomerular basement membrane (type II membranoproliferative glomerulonephritis; MPGN II). Hypocomplementemia (decreased complement levels) as a result of C3 NeF was associated with meningococcal disease,[157] as was a transient complement deficiency caused by post-streptococcal glomerulonephritis.[158]

Activation of complement plays a role in the pathogenesis of several diseases including paroxysmal nocturnal hemoglobinuria (PNH), atypical hemolytic uremic syndrome (aHUS), C3 glomerulonephritis, dense-deposit disease (DDD, or MPGN II), age-related macular degeneration (AMD), and rheumatoid arthritis.[159] Targeted complement inhibition may represent an attractive therapeutic target in such disorders. A monoclonal antibody called eculizumab that prevents C5 cleavage is approved for the treatment of PNH and has also been used in aHUS and shiga-toxin associated HUS. In a study of eculizumab in 195 persons with PNH, two episodes of meningococcal disease were reported, which translated to an incidence of 4.2 episodes per 1000 patient-years.[160] The first case was caused by a group B isolate that was not covered by the quadrivalent (groups A, C, W, and Y) vaccine, while the other patient, who received a group A/C vaccine, was infected with a group W or Y isolate (specific serogroup designation was not performed in the patient's country). A case of group W meningococcal sepsis was also reported in a 19-y-old woman with factor H mutation-associated aHUS who received eculizumab to prevent recurrence of aHUS following her third renal transplant.[161,162] This individual was on several immunosuppressants and responded very poorly to an unconjugated quadrivalent meningococcal polysaccharide vaccine, which may have contributed to her susceptibility to infection. Collectively, a mounting body of evidence suggests that individuals with acquired complement defects secondary to complement consumption states may be at a higher risk for invasive meningococcal infections.

Complement Evasion Strategies Used by *Nm*

Nm benignly colonizes the nasopharyngeal mucosal epithelium of up to 40% of the population without causing any signs of disease or sepsis; for this unusual pathogen, disease is the exception rather than the rule. A complex interplay exists between the ability of a specific colonizing strain of *Nm* to cause disease and the susceptibility of a specific host to infection. The host factors that predispose one to meningococcal disease are disproportionately clustered in complement system proteins, as discussed above, and it is apparent that a properly functioning complement system is essential to combat meningococcal infection. The following section, will describe some of the virulence factors that allow *Nm* colonizing the nasopharynx to invade the bloodstream and cause life threatening sepsis. Given the fundamental role of complement-mediated killing in combating *Nm* infections, the focus will be on those factors that specifically allow *Nm* to avoid killing by the complement system. Meningococcal virulence determinants that promote complement evasion are summarized in **Table 3**.

Capsule and lipooligosacchride in serum resistance

The ability of *Nm* to survive killing by the innate immune system and cause disease is highly dependent upon its ability to express capsular polysaccharide and lipooligosaccharide. Compelling evidence supporting the central role of these two molecules was revealed in a genome wide screen of more than 3000 mutants analyzed for their ability to resist killing by 25% NHS; of 18 mutants identified as highly sensitive to killing, all 18 had defects in either capsule or LOS biosynthesis.[163]

Capsule

Capsule is the major virulence determinant of *Nm* and essentially all invasive strains of *Nm*, isolated from individuals without underlying immune defects, elaborate capsular polysaccharide. Capsular polysaccharide is composed of repeating saccharide units linked by glycosidic bonds and the composition of the meningococcal capsule is used to divide *Nm* into 12 immunologically distinct groups; the overwhelming majority of disease is caused by just 6 of these groups (A, B, C, W, X, and Y). The group B, C, W, and Y capsules all contain sialic acid, a compound known to inhibit complement activation on host cells. The group B and C capsules are α(2,8)- and α(2,9)-linked homopolymers of sialic acid while the W and Y capsules contain alternating sequences of either D-galactose (W) or D-glucose (Y) and sialic acid. The group A and X capsules do not contain sialic acid and are composed of repeating units of *O*-acetylated (α1'6)-linked *N*-acetyl-D-mannosamine-1-phosphate and (α-1'4)-linked *N*-acetylglucosamine 1-phosphate, respectively. The A, C, W, and Y polysaccharides can also be *O*-acetylated, but this does not appear to affect the serum resistance properties of the capsule. Loose geographic biases are associated with capsule distribution; groups B and C historically predominate in industrialized countries, although the prevalence of disease caused by group Y has been increasing in North America. Groups W and X are often found in the meningitis belt of west Africa while group A strains are highly represented in sub-Saharan Africa. The reasons for these distributions are not understood, but may reflect host immune factors as well as differences in the strains ability to evade the immune system.

Capsular polysaccharides are thought to benefit bacteria by preventing desiccation and facilitating transmission, however recent studies with *Nm* refute this theory.[164] The most fundamental role of the meningococcal capsule seems to be preventing complement-dependent killing. *Nm* that lack a capsule

Table 3. Meningococcal complement evasion strategies

Virulence factor	Role(s) in complement evasion
Polysaccharide capsule	Expression is required for serum resistance[168,265,266]
	Upregulation enhances resistance[165]
	Upregulation decreases deposition of C3b[165]
	Regulation of the AP:
	Group A: no effect on C3b deposition[33]
	Groups B and C: decreases C3b deposition[33,165,166]
	Groups W and Y: enhances C3b deposition; directly binds to C3b[33]
	Molecular mimicry prevents generation of effective Ab
	Group B polysaccharide capsule is identical to human neural-cell adhesion molecule (NCAM-1)[205]
	Target for generation of blocking Abs[219-221,224,267,268]
	Diverts MAC to non-bactericidal sites[37]
	Anti-phagocytic[269]
Lipooligosaccharide (LOS)	Target for deposition of complement C3 and C4[270,271]
	Phase variable expression:
	Thwarts effective Ab generation
	Diverts deposition of complement C3 and C4[172]
	Molecular mimicry: hinders effective Ab production:
	LNT LOS mimics glycosphingolipids and glycolipid antigens on human erythrocytes and granulocytes such as paragloboside (LNT-ceramide) or the human erythrocyte I antigen precursor[272,273]
LOS sialic acid	Regulates the AP[168,181,183,274]
	Enhances binding of fH (SCRs 18–20) to deposited C3b[183]
	Molecular mimicry hinders effective Ab production:
	Sialylated LNT LOS mimics sialylparagloboside or sialyllactosamine (human erythrocyte I antigen)[272,273]
	Increases resistance to opsonophagocytosis[269]
Porin A (PorA)	Binds C4BP under hypotonic conditions[185]
	Phase variable: downregulation may offset Ab efficacy
Porin B (PorB2 and PorB3)	PorB2: fH dependent regulation of the AP[196]
	Expression of PorB2 enhances serum resistance as compared with isogenic mutant expressing PorB3[196]
fHbp	Binds to human fH (SCR6–7)[244,275]
	Regulates the AP[30,183,187,276]
	Expression enhances serum resistance
NspA	Binds to human fH (SCR6–7)[186]
	Regulates the AP[183,186]
	Expression enhances serum resistance[186]
Opc	Binds to vitronectin, inhibits the terminal complement pathway and enhances serum resistance[200,277]
Msf	Binds to vitronectin, inhibits the terminal complement pathway and enhances serum resistance[200]
H.8 and Laz	AAEAP motifs are target for generation of blocking Abs[225]
IgA Protease	Cleaves IgA, hinders Ab binding and function[228]
	May play role in biofilm formation[278]

Table 3. Meningococcal complement evasion strategies (continued)

Virulence factor	Role(s) in complement evasion
Opa	Target for deposition of complement[271]
	Phase variable expression: "moving target" for effective Ab generation and function
	Ng Opa bind vitronectin and enhance serum resistance[279]
Pili	Phase variable expression and antigenic variation impedes broadly-reactive Ab generation and Ab function
	May bind to the membrane complement regulator CD46[203]
NHBA	Binds heparin and improves serum resistance of unencapsulated meningococci[280]
Blebs or OMVs	Divert complement activation and antibody binding away from live intact bacteria
	Potentiate sepsis by increasing LOS (endotoxin) in bloodstream[231]
Biofilms	Render bacteria resistant to immune defenses
	Nm factors that may be important for biofilm formation include; type IV pili, extracellular DNA, HrpA, NalP, IgA protease, and NHBA[278]

are highly sensitive to killing by human sera when compared with encapsulated counterparts. Further, the ability to resist serum killing is enhanced in strains that express increased amounts of capsular polysaccharide; group C *Nm* strains that contained an IS 1301 sequence that upregulated capsule synthesis and transport were highly resistant to killing by antibodies elicited by the MenC conjugate vaccine.[165] Despite a general acceptance of the key role of capsular polysaccharide in downregulating complement activation, the exact mechanisms involved remain unclear. On host cells, sialic acid enhances the interaction of fH with C3b and limits AP activation. Although the sialic acid containing groups B and C capsules downregulate the AP,[33,166] the mechanism may not involve enhancement of fH-C3b interactions; colominic acid, a similar α(2,8)-linked sialic acid derived from *E. coli* K1 capsule, does not enhance the interaction of fH with deposited C3b.[167] Complement regulation by *Nm* is not restricted to strains that express sialic acid containing capsules; the group A capsule that is devoid of sialic acid confers increased resistance to killing by serum.

Several studies have investigated regulation of the AP by the meningococcal capsule but the results have been conflicting. Enhanced AP activation (increased C3b deposition) was reported in a sialic acid-deficient mutant (chemically-induced) of group B strain B16B6[166] but similar levels of C3b were detected on unencapsulated and encapsulated group B strain B1940.[168] The highly serum resistant group C *Nm* strains with IS 1301 mediated upregulation of capsule expression more effectively regulated the AP and decreased C3 deposition. The molecular mechanisms of complement regulation by capsule are likely complex and influenced by heterogeneity in strain background, including variable expression of additional factors that regulate activation of the AP (see below). Using isogenic strains that were devoid of all other known AP regulating molecules and that represented five different groups, AP inhibition by the group B and C capsules was confirmed.[33] Unexpectedly, the W and Y capsules, both of which contain sialic acid, enhanced AP activation and these capsules themselves served as sites for deposition of C3b.[33] The group A capsule did not affect AP-mediated C3b deposition. These studies indicate that the mechanism of complement regulation by capsule varies with the chemical composition of each capsule. Other studies have reported that capsular polysaccharides prevent proper insertion of MAC[37] and may downregulate the CP by altering the binding properties of antibodies directed against surface epitopes. Elucidation of the diverse factors that impact complement regulation by *Nm* will aid in defining how each capsule type interacts with complement.

LOS structure

LPS is the primary component that makes up the outer leaflet of the outer membrane of gram-negative bacteria. It plays a major role in membrane stability and is responsible for several of the deleterious clinical features of gram-negative sepsis. The level of LPS circulating in the bloodstream correlates with the morbidity and mortality of meningococcal bacteremia.[109] LPS is composed of a hydrophobic lipid A (endotoxin) anchor, a hydrophilic inner core that often contains heptoses and KDO (3-deoxy-D-manno-oct-2-ulosonic acid) and a repeating O-antigen side chain composed of oligo- and polysaccharides. Neisserial LPS differs from that of *Escherichia coli* in that the "O-antigen" does not contain repeating units and is therefore commonly referred to as lipooligosacchride (LOS). Many of the genes encoding the glycosyltransferases that are responsible for synthesis of neisserial LOS are subject to high frequency (1:1000) reversible "on"/"off" phase variation, which generates LOS structural diversity. Based on monoclonal antibody reactivity, 12 antigenically distinct *Nm* LOS structures (L1–L12) have been defined and form the basis for the *Nm* "immunotyping" system.[169] An investigation of LOS immunotypes of epidemiologically related case (i.e., recovered from the blood or cerebrospinal fluid; n = 36) and carriage (isolated from the nasopharynx of asymptomatic individuals; n = 76) isolates associated with a community outbreak of group B meningococcal infection in Glouscestershire, UK revealed that 97% of case isolates expressed the L3,7,9 LOS (sialylated lacto-*N*-neotetraose extension from HepI), while only 24% of carriage strains expressed this LOS immunotype. Nasopharyngeal isolates most frequently expressed the L1,8,10 immunotype, which comprises a lactose extension from HepI that cannot

be sialylated.[170] Expression of the L3,7,9 LOS in this "clone" facilitated invasion of the bloodstream in an infant mouse intranasal infection model.[171]

Neisserial LOS is a site for deposition of both C3b and C4b and subtle differences in LOS structure may impact serum resistance by altering the availability of sites for complement deposition. As an example, *Nm* LOS expressing phosphoethanolamine (PEA) on the 6-position of the second heptose (HepII) of the core region, are rarely isolated from patients (<30% of invasive isolates). C4b derived from the C4A isoform preferentially forms amide bonds with PEA at the 6-position relative to the 3-position and strains bearing 6-PEA are more sensitive to killing by serum, in vitro, than strains that express PEA on the 3-position of HepII.[172] Modulation of complement activation could drive selection of specific LOS immunotypes in nature.

The terminal lacto-*N*-neotetraose (LNT) epitope of LOS can be modified with sialic acid by the enzyme α-2,3-sialyltransferase (Lst); cytidine monophospho-*N*-acetylneuraminic acid (CMP-NANA), the donor molecule for sialic acid, is produced endogenously by strains of *Nm* that contain sialic acid in their capsule and can be scavenged from the environment by all strains. The importance of LOS sialylation in meningococcal serum resistance has been alluded to in many studies, however the relative importance and precise mechanisms of enhancing serum resistance remain ambiguous due to conflicting reports. For example, isogenic mutants of B1940 (group B) and C2120 (group C) that lacked the ability to sialylate LOS were more sensitive to killing by serum than the parent strain, but the same was not true of similar mutants constructed in group B strain MC58.[173] Early studies, like these, may have been complicated by the variable expression of other factors that regulate the AP of complement that were not known at the time (such as fHbp and NspA—see below) or the overriding effects of capsule may have masked regulation by LOS sialic acid.

In *Ng*, LOS sialylation unequivocally enhances resistance to killing by human serum and the mechanism is well characterized; sialylation of *Ng* LOS decreases binding of select antibodies[174] and also enhances binding of the complement regulator fH to porin B,[175] which allows for regulation of the CP and AP, respectively and converts serum sensitive strains to a serum-resistant phenotype. *Ng* express higher levels of sialyltransferase activity than *Nm*,[176] and *Ng* that are unable to sialylate LOS (*lst* mutants) or *Ng* with decreased levels of sialyltransferase activity are attenuated (relative to wild-type strains) in a mouse model of gonococcal infection.[177,178] In contrast to *Ng*, sialylation of meningococcal LOS does not promote high affinity binding of human fH to porin B[179] and *lst* mutants, lacking the ability to sialylate LOS, are able to cause bacteremia in infant rats (both wild-type and human fH transgenic) to the same extent as the wild-type strains.[180] Lactate permease (*LctP)* mutants of *Nm* that are defective in sialic acid biosynthesis were attenuated during bloodstream infection of wild-type infant rats, but were not attenuated in C3-deficient mice.[181] This observation highlights the role of C3 in meningococcal virulence; however, the requirement for sialic acid in both capsule synthesis and LOS sialylation must be considered when interpreting this result.[181] Sialic acid on neisserial LOS (unlike capsular sialic acid) may instead function like sialic acid on host cells and subvert the AP by enhancing interactions of fH with surface bound C3b.[182] LOS sialylation enhanced binding of fH, specifically fH SCRs 18–20, to C3b deposited on the *Nm* surface.[183] These studies used mutants of H44/76 that lacked other known ligands for human fH and the relative contributions of these factors is discussed below. Although LOS sialylation plays a key role in virulence and serum resistance, the isolation of strains that lack the LNT epitope from human disease suggests that LOS sialylation is not required for virulence.[171]

Binding of host complement regulatory molecules by *Nm*

One of the most common strategies employed by bacterial pathogens to evade killing by complement is to highjack host complement regulatory proteins and use these molecules to downregulate complement activation.[184] *Nm* recruits numerous complement regulators to block activation of complement at several key steps.

C4 binding protein

Protective immunity is correlated with the presence of anti-meningococcal antibodies that activate the CP of complement. C4 binding protein (C4BP) is a negative regulator of the CP that functions to inactivate the CP C3 convertase and to convert active C4b to inactive forms. *Nm* porin A has been reported to bind to C4bp; however, strong binding occurred under only under hypotonic conditions.[185] *Nm* uses several other mechanisms such as molecular mimicry, phase variation and antigenic variation (discussed below) to subvert CP killing and the role of direct binding of C4BP to *Nm* remains to be elucidated.

Factor H

A pivotal role of the AP is to activate C3; all three complement pathways converge at the level of cleavage of C3 and the C3 amplification loop of the AP contributes to maximal functioning of antibodies.[30] The AP is required for efficient killing of *Nm* and as such meningococci have evolved numerous redundant systems specifically aimed at disarming the AP by capturing host fH. fH binding protein (fHbp) and neisserial surface protein A (NspA) are two key proteins expressed on the surface of meningococci that bind to fH and regulate the AP.[186,187] Expression of either of these proteins decreases deposition of C3b and increases resistance to killing by human sera. fHbp is a ~28 kDa lipoprotein composed of two extracellular domains, an elongated flexible β-sheet and a C-terminal eight-stranded β-barrel domain, that are connected to each other by a flexible linker and tethered in the outer membrane by an N-terminal lipid anchor.[188,189] Based on its amino acid sequence, fHbp is divided, into either three variant groups[190] of two sub-families[191] depending on the classification system used; all variants bind fH and regulate the AP. fHbp is the target of bactericidal antibodies and is a key component of a several meningococcal vaccines being evaluated in clinical trials. NspA is a ~17 kDa integral membrane protein with an 8-stranded anti-parallel β-barrel and 4 predicted short (<14 amino acids) surface exposed loops[192] that is highly conserved across *Nm* strains. In vitro binding of fH to NspA is impacted by other components on the *Nm* surface; binding increases when capsular polysaccharides are downregulated or when the LNT LOS epitope is either

sialylated or not expressed.[186] The level of expression of both fHbp and NspA varies widely among *Nm* strains; some strains express high levels of fHbp and low levels of NspA while others display the opposite pattern.[183] It is clear that expression level of each protein correlates with the level of fH binding and both proteins are predicted from microarray analysis to be upregulated in human blood.[193,194] The relative contribution of each ligand to fH binding and AP regulation in vivo is likely complex and dependent upon several factors including expression level, LOS diversity and level of capsule expression.[183] As noted above, sialic acid on *Nm* LOS functions similar to sialic acid on host cells and subverts the AP by enhancing interaction of fH with surface bound C3b. Maximal inhibition of the AP on group B strain H44/76 was only observed when all three AP downregulating mechanisms (fHbp, NspA, and LOS sialylation) were present.[183]

The functional redundancy of these three fH binding mechanisms was illustrated by meningococcal bacteremia studies in infant rats. *Nm* is an exclusively human pathogen and fHbp and NspA show maximal binding to human fH.[195] In contrast, LOS sialylation enhancement of fH binding to C3b was independent of host species. LOS sialylation enhanced binding of rat fH to C3b on the *Nm* surface and effectively regulated rat complement.[183] Bacteremia with *Nm* strain H44/76 is enhanced in transgenic rats that express human fH.[180] Mutants lacking fHbp only, both fHbp and NspA or *lst* only (unable to add sialic acid to LOS) all successfully caused bacteremia in fH Tg rats.[180] Only triple mutants of H44/76, lacking all three fH binding methods, fHbp, NspA, and LOS sialic acid, were unable to cause bacteremia in fH Tg infant rats.[180] These studies, conducted with encapsulated H44/76 (group B), not only demonstrate the ability of each of these mechanisms to independently subvert the AP in the presence of human fH, but also highlights the inability of sialic acid in the group B polysaccharide capsule to recruit human fH to successfully regulate the AP in human fH transgenic rats.

Invasive isolates of *Nm* that do not express fHbp (due to either deletion or mutation of the *fhbp* gene) or appear to bind human fH have been described. This suggests that additional undiscovered mechanisms for regulating the AP of complement may exist. *Nm* expresses two distinct porin molecules, PorA and PorB. PorB can further be divided into two sub-classes; PorB2 and PorB3, which are mutually exclusive and are expressed from alternate alleles (*porB2* and *porB3*) at the *porB* locus. Recently, an analysis of AP regulation by *Nm* that were genetically constructed to lack all known AP regulating mechanisms (capsule, fHbp, NspA, and LOS sialic acid) revealed that PorB2 could also regulate the AP.[196] Regulation of the AP by PorB2 was dependent upon human fH.

Vitronectin

The membrane attack complex (MAC) plays a key role in combating neisserial infections and persons deficient in terminal complement components have greatly increased incidence of meningococcal disease. Vitronectin is an important extracellular matrix molecule with numerous functions, including complement regulation as described above. The association of vitronectin with the soluble terminal complex inhibits insertion of MAC into the host cell membranes and prevents undesired lysis. Many bacterial pathogens bind vitronectin[184] and use this molecule to enhance serum resistance. *Nm* bind vitronectin and interestingly, the concentration of circulating vitronectin is diminished during meningococcal sepsis. The low vitronectin levels during acute infection were likely due to a combination of factors including hemodilution, extravasation and its incorporation into terminal complement or vitronectin–thrombin–antithrombin complexes generated as a result of complement activation; there was no association between serum vitronectin levels measured in patients who recovered compared with healthy controls.[197] Two meningococcal surface proteins have been reported to bind to the activated form of vitronectin. The opacity protein, Opc is a 10-stranded β-barrel with extracellular loops[198]; expression of *opc* is phase variable and not all *Nm* strains contain the *opc* gene. 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. Vitronectin–Opc interactions also decrease MAC deposition and enhance resistance to complement-mediated killing.[199] The meningococcal surface fibril, Msf (previously, NhhA and Hsf) is an adhesin predicted to form trimeric fibrils of ~45 nm. OpcA and Msf function independently and binding of vitronectin to either protein enhances serum resistance.[200] Consistent with the function of vitronectin, expression of Opc and Msf reduces MAC deposition but does not affect deposition of C3. Expression of Opc and Msf varies among meningococcal strains and expression of both proteins simultaneously increases serum resistance relative to expression of either protein independently.

Properdin

Properdin is the only known positive regulator of the AP that functions by stabilizing the AP C3 convertase. Properdin deficiencies are associated with an increase in meningococcal disease and this likely reflects the important role of the AP in controlling *Nm* infections. Purified, commercially available properdin was reported to bind directly to *Ng* and activate the AP.[22] Commercial properdin preparations that have undergone freeze-thawing cycles often contain aggregates that could result in non-specific binding to surfaces.[201,202] Freshly fractionated "native" forms of properdin did not bind either to *Ng* or to *Nm* directly, suggesting that the role of properdin in combating neisserial infections appears to be through the conventional mechanism of augmenting AP-dependent C3 deposition.[27]

Membrane-bound complement regulators

Nm can interact with host membrane-associated complement regulatory molecules and these interactions may be important for virulence. CD46 is found on most nucleated cells and serves as a cofactor in the factor I-mediated cleavage of C3b and C4b to inactive forms. The type 4 pili of *Ng* binds to CD46 but the role of *Nm* type 4 pili in binding to CD46 is questionable. In one study, a small increase in mortality was seen when human CD46 transgenic mice were challenged intranasally with piliated meningococci while animals challenged with nonpiliated bacteria showed 100% survival.[203] In separate experiments where bacteria were administered intraperitoneally, mice expressing human CD46 were also more susceptible to infection with *Nm* than wild-type mice, but this was independent of the piliation status of

the bacterium.[203] The role of CD46 as a receptor for *Nm* has been questioned because CD46 is expressed mostly on the basolateral surface of epithelial cells.[204] The ability of *Nm* to interact with CD46 and other membrane bound complement regulators is less well studied and merits further investigation.

Evasion of antibody-mediated CP killing

Protective immunity is correlated with the presence of anti-meningococcal antibodies that activate complement. In addition anti-*Nm* antibodies may play a role in opsonophagocytosis. Blocking the formation and/or function of protective antibodies is an effective way to counter CP activation and limit opsonization. *Nm* benefit from a diverse set of mechanisms to achieve this purpose.

Molecular mimicry

Host compounds are not immunogenic and under normal circumstances molecular mimicry of host compounds is an effective method used by *Neisseria* to block the generation of effective (CP activating) bactericidal antibodies. The neisserial surface is rich with compounds that mimic those found in the host. The group B capsule, a repeating unit of α(2,8)-linked sialic acid is identical to the human neural cell-adhesion molecule,[205] NCAM and, as a result, is poorly immunogenic. Similarly the carbohydrate moieties of LOS mimic host carbohydrates present in glycosphingolipids of host cells; as an example the LNT epitope of LOS is identical to the human I erythrocyte antigen.[206]

Phase and antigenic variation

The extracellular surface of *Nm* is extremely antigenically diverse and this diversity successfully forms a moving target for effective humoral responses and thereby allows *Nm* to evade killing by the CP. Phase variation and antigenic variation are two key processes that drive neisserial diversity. Phase variation is a reversible high frequency (1:1000) method for turning the expression of genes "on" or "off".[207] Phase variation can also occur in promoter regions and result in up or downregulation of gene expression rather than true on/off switching.[207] Neisserial phase variation most commonly involves strand slippage at repetitive DNA sequences during DNA replication and this impacts gene expression. Numerous genes, including those involved in the synthesis of LOS, capsule and pilin are well known to be controlled by phase variation. Conservative estimates based on genome wide analysis have identified over 50 neisserial genes that are very likely to be phase variable; half of these are surface proteins or enzymes that modify the antigenic make-up of the surface (i.e., LOS biosynthesis genes). Phase variation can result in the presence/absence of an antigen, alter levels of antigen expression, or it can result in antigenic variation when the gene controls synthesis of a larger macromolecule. Phase variation of the glycosyltransferases that synthesize LOS is an example of the latter.[208] In addition to thwarting antibody production, phase variation also alters expression of common targets for complement deposition, including LOS and the opacity proteins.

Antigenic variation is a distinct process by which bacteria generate multiple antigenic versions of the same protein, at high frequency, through non-reciprocal DNA recombination (gene conversion).[209] Neisserial pili are essential for virulence and anti-pilin antibodies are effective at blocking infection, however, the pilin gene is subject to antigenic variation and this effectively blocks successful immune responses. Pilin antigenic variation results from recombination between a pilin expression locus (PilE) and one of multiple pilin silent or storage (PilS) loci. The neisserial chromosome harbors ~8 to 19 *pilS* loci and variation in pilin expression effectively combats the formation of useful anti-pilin antibody. Genomic analysis indicates that other *Nm* genes are subject to similar antigenic variation.

Blocking Abs

While the genesis of an antibody response against a colonizing or invading pathogen is often protective, in some instances the elicited response can decrease killing by otherwise bactericidal antibodies and enhance the susceptibility of an individual to infection. Such antibodies, sometimes called "blocking antibodies", were first reported in1894 by Pfeiffer[210] who noted that animals given excess immune serum were more susceptible to infection when challenged with organisms against which the immune serum was raised.[211] Blocking antibodies play an important role in pathogenesis and have been described in relationship to infections with *Brucella abortus*,[212] *E. coli*,[213] *P. aeruginosa*,[214] non-typhoidal *Salmonella* infection in HIV-infected individuals,[215] and the pathogenic *Neisseria*. In *Ng*, the presence of blocking antibodies that are directed against the reduction modifiable protein (Rmp) are correlated with increased susceptibility to infection.[216] A longitudinal study of 243 female commercial sex workers in Nairobi showed that women who possessed antibodies against Rmp were more likely to experience repeated infections compared with women without anti-Rmp antibody (adjusted odds ratio 3.4).[216]

Nm is no exception to the recurring theme of subversive antibodies, however the role of anti-Rmp (class 4 in *Nm*) antibodies in blocking killing of *Nm* by bactericidal antibodies is less clear. One study detected natural anti-class 4 "blocking antibodies" in serum from one individual, but anti-class 4 antibodies induced by vaccination with a trial meningococcal outer-membrane vesicle vaccine did not exhibit blocking properties.[217] In *Nm* blocking antibodies are directed against capsular polysaccharides and a pentapeptide repeat motif (AAEAP) found in two meningococcal lipoproteins called H.8 (or Lip) and Laz and is discussed below.

Thomas and his colleagues observed that convalescent sera from some patients recovering from meningococcal infection were less effective at killing meningococci than sera collected during the acute phase of infection. These sera contained IgA that blocked killing by otherwise bactericidal normal human sera.[218] In a subsequent study, IgA purified from human serum on days 12, 33, and 27 following infection with groups B, C, and Y meningococci, respectively, blocked complement-mediated bacteriolysis by the same sera. Blocking depended on the ratio of lytic to blocking antibody and was strain specific.[219] Bactericidal IgG was more readily blocked than otherwise cidal IgM.[219] In a separate study of 28 military recruits with meningococcal disease, 24 lacked bactericidal activity; removal of IgA from these 24 sera uniformly enhanced the bactericidal activity of IgM present in the same sera.[220] IgA1 directed against *Nm* group C

polysaccharide blocked the bactericidal activity of IgG; blocking was not because of competitive inhibition of IgG binding and did not require the Fc region of IgA1 for function.[221] An analysis of 47 sera obtained from individuals from the Sudan showed that 16 (34%) had bactericidal titers <1:4. Eight of these non-bactericidal Sudanese sera had total anti-capsular antibody concentrations >15 μg/ml and seven of the eight sera had anti-capsular IgG concentrations ≥10 μg/ml. A possible reason cited for the lack of bactericidal activity of these sera even in the face of high anti-capsular IgG titers was the presence of a high proportion of anti-group A capsular IgA antibody that may have served to block killing by otherwise bactericidal IgG or IgM antibodies.[222] Similarly, anti-group A capsule "blocking" IgA was cited for the lack of bactericidal activity of select immune sera in a previous report.[223] Selander et al. showed that vaccination of a C2 deficient individual elicited anti-group W capsule IgG that blocked alternative pathway-dependent killing of group W meningococci, probably by limiting the amount of C3 deposited on group W capsule.[224] Blocking antibodies have also been reported in the sera of normal individuals with no history of meningococcal disease. IgG antibodies directed against the AAEAP pentapeptide repeat motifs present in H.8 (Lip) and Laz could block killing by select antibodies, including those directed against the group B meningococcal antigen, fHbp.[225]

The mechanism of blocking appears to vary considerably with each specific blocking antibody. Anti-*Ng* Rmp blocking antibodies are associated with increased C3 and C9 deposition on the bacterial surface and the lack of killing is presumably because complement components are diverted to non-bactericidal targets.[226] Anti-H.8 blocking antibodies reduced C4b deposition; blocking required the Fc portion, evidenced by the observation that $F(ab')_2$ fragments lost the ability to block killing.[225] In contrast, $F(ab')_2$ and Fab derived from anti-capsule blocking antibodies prevented killing to the same extent as the intact antibody.[221] Regardless of their mechanism of action, blocking antibodies may reduce the efficacy of anti-meningococcal vaccine antibodies. Defining the targets for blocking antibodies and eliminating these molecules from vaccine preparations may promote vaccine efficacy.

IgA protease

IgA is produced locally on mucosal surface and may be a first line of defense against invading pathogens.[227] Many pathogens that colonize the mucosal surface, including *Nm* produce secreted IgA proteases that cleave IgA1 at hinge region and remove the Fc portion. It has been postulated that the remaining Fab fragments may bind or remain bound to the organism, however other studies have reported low affinity of the remaining Fab portion of IgA.[228] The precise biological significance of IgA protease remains elusive.

Other mechanisms of complement evasion

Biofilms

Biofilms are a complex organized community of bacteria surrounded by a protective matrix often made of exopolysaccharides and DNA; the bacteria within are living, but not actively growing. Biofilms are known to protect bacteria from a variety of environmental stresses including antimicrobial agents and host clearance mechanisms. *Nm* can colonize the nasopharyngeal epithelium of humans for extended periods of time and must have strategies to evade killing and clearance by the mucosal immune system. Immunological staining techniques have detected microcolonies of *Nm* under the epithelial surface that escape detection using traditional culture methods.[229] *Nm* can reportedly form biofilms or biofilm-like structures in vitro and may form similar structure in vivo.[229,230] The contribution of meningococcal "biofilms" to evasion of the local immune system, prolonged carriage and/or progression from carriage to invasive disease remains to be defined.

Decoy

Nm release large amounts of outer membrane vesicles or "blebs" into the bloodstream.[231] These membrane blebs, which contain membrane proteins and LOS, present a challenge to the immune system in several ways. Blebs can act as decoys by activating complement at futile locations and by directing otherwise effective responses away from intact bacteria. In addition, blebs increase the amount of LOS (endotoxin) in bloodstream and thus increase pro-inflammatory responses and concomitant damage.

Prevention of Meningococcal Disease

Meningococcal sepsis is characterized by a rapid onset of symptoms; death can occur within a few hours and survivors are often left with loss of digits, limbs, or persistent neurological sequelae. For these reasons, emphasis should be placed on prevention through vaccination.

An overview of meningococcal vaccines

Polysaccharide-based vaccines

Gotschlich, Goldschneider, and Artenstein were the first to extract meningococcal polysaccharides and demonstrate their immunogenicity;[232] their efforts in collaboration with the Merieux Institute led to the production of the first effective meningococcal vaccine in 1972. A tetravalent unconjugated meningococcal polysaccharide vaccine was licensed for use in the United States in 1981. Although generally safe and effective, unconjugated polysaccharide vaccines do not recruit T-cell help and as a result have several limitations including short-lived protection and a lack of efficacy in young children (<2 y of age). Further, repeated doses of unconjugated polysaccharides may induce immunologic hyporesponsiveness and should be avoided.[233-236] Conjugation of polysaccharides to proteins results in vaccines that recruit T-cell help and induce higher anti-polysaccharide antibody responses that persist longer and induce immunological memory. Currently, several meningococcal conjugated polysaccharide vaccines are available in various parts of the world; tetravalent (groups A, C, W, and Y) conjugate polysaccharide vaccines (Menactra, Menveo, and Nimenrix), a bivalent group C/Y meningococcal vaccine in combination with *Haemophilus influenzae* type b polysaccharide (MenHibrix) and several monovalent group C polysaccharide-protein conjugate vaccines (e.g., MenC, Meningitec, Menjugate, and NeisVac-C). A monovalent group A conjugate vaccine (MenAfriVac) is being

widely administered in the Sub-Saharan meningitis belt of Africa.

Protein based group B vaccines

Group B strains of *Nm* cause a substantial proportion of disease worldwide yet none of the polysaccharide vaccines described above provide protection against group B strains. The structural similarity of group B polysaccharide with host tissue molecules such as neural-cell adhesion molecule (NCAM-1) has precluded its use in vaccines.[205] Outer membrane vesicle (OMV) vaccines derived from group B strains have been used with success to control epidemics caused by specific strains in Norway,[237] Cuba,[238] and New Zealand.[239,240] Currently, OMV vaccines are most effective at controlling epidemics caused by the strain used to produce the vaccine but efforts to broaden the protective properties of OMV are being investigated—as an example, multiple PorA may be incorporated into a single vaccine preparation.[241]

To circumvent the limitations associated with polysaccharide antigens and to develop a vaccine that would be effective against group B strains of *Nm*, research has focused on the identification of suitable protein antigens and two promising vaccines have recently been developed. Novartis used a "reverse vaccinology" approach[242] to identify 5 candidate protein antigens; fHbp, NHBA (neisserial heparin binding antigen; previously GNA2132), NadA (neisserial adhesin A), GNA1030, and GNA2091.[243] These 5 antigens, in the form of 3 recombinant proteins, were combined with the New Zealand OMV vaccine preparation (to enhance immunogenicity) and this vaccine, called Bexsero, has recently been licensed for clinical use in Europe. Pfizer, using a membrane fractionation approach, also identified fHbp as a potent immunogen that elicited anti-meningococcal bactericidal antibodies. A bivalent fHbp vaccine formulation that comprises two antigenically distinct lipidated fHbp molecules has undergone clinical trials with promising results.[188] Although licensed as vaccines against group B meningococci, these newer protein-based vaccines are likely to be active against diverse groups.

Immunogenicity of antigens that bind to host proteins

The fH binding protein, fHbp is a key antigen in both protein based vaccine formulations. The effects of human fH, which is present in serum at high concentrations (300–600 μg/ml) and binds to fHbp, on the immunogenicity of fHbp based vaccines could not be assessed in preclinical (non-human) studies because fHbp only binds to human fH.[195] The co-crystal structure of human fH domains 6 and 7 in a complex with fHbp revealed a large area of interaction and the fH binding region of fHbp overlaps with the binding sites of several anti-fHbp bactericidal monoclonal antibodies.[244] To further address this question, BALB/c mice that expressed full-length human fH were used; two separate studies demonstrated impaired immunogenicity of fHbp in human fH transgenic mice compared with wild-type mice.[245,246] Immunization of transgenic animals with a mutant fHbp protein that did not bind to human fH restored immunogenicity.[245,246] These data suggest that binding of host complement inhibitors to vaccine antigens may impair their immunogenicity. fHbp in Bexsero is present as a fusion protein in complex with genome-derived neisserial antigen 2091 (GNA2091). Whether human fH interacts with fHbp present in Bexsero to attenuate its immunogenicity is not clear, but merits consideration in future vaccine design.

Immunization of complement-deficient individuals

The Advisory Committee on Immunization Practices (ACIP) recommends that all persons at a high risk for meningococcal infection, including complement-deficient individuals, receive a primary vaccination series with a tetravalent conjugate capsular polysaccharide vaccine 8–12 weeks apart, followed by booster dose every 3–5 y; the regimens are dependent on the age of the individual and the reader is referred to recent ACIP guidelines.[136] While most terminal complement deficient persons elicit primary antibody responses comparable to normal individuals, antibody responses in other high risk groups such as persons with anatomic or functional asplenia or advanced HIV infection may be impaired, which has prompted recommending the 2-dose primary vaccination regimen for high-risk groups.[136] The efficacy of these vaccines in complement deficient individuals has not been extensively evaluated, but anti-capsular antibodies elicited by vaccination would be expected to enhance CP-mediated complement recruitment in AP-deficient patients and facilitate antibody-mediated AP recruitment in persons lacking CP components.

Complement-dependent bactericidal activity is the accepted surrogate of protection against meningococcal infection but individuals with LCCD depend on opsonophagocytosis for protection. There is good evidence for opsonophagocytic protection by specific antibody and this provides rationale for immunization of LCCD patients. Antibody titers against groups A and C polysaccharide were similar in 8 LCCD persons, 11 of their family members and 7 unrelated normal individuals.[247] Antibody responses declined more rapidly in complement-deficient persons but the elicited anti-capsular antibodies promoted opsonophagocytic killing. Similarly, enhancement in opsonophagocytic activity was reported in three C7-deficient siblings following administration of the quadrivalent polysaccharide vaccine.[248] Rates of meningococcal infection in 18 LCCD patients immunized with the quadrivalent polysaccharide vaccine were ~4.5 times lower compared with rates in the same individuals over the decade leading up to immunization and also when compared with rates in 8 LCCD individuals who did not receive the vaccine.[249] Similar results were reported by Platonov and colleagues who studied the efficacy of the quadrivalent polysaccharide vaccine in Russian patients with LCCD who had experienced between one and five episodes of meningococcal infection.[94] Of 45 such patients, 31 were immunized with a quadrivalent meningococcal polysaccharide vaccine, with a booster dose administered after 3 y. Adequate antibody responses were documented in these individuals. Six new episodes of meningococcal infection occurred in 4 of the 31 patients given the vaccine. In contrast, among the 14 unimmunized LCCD patients there were 6 episodes of infection in 6 patients. A significant survival benefit in vaccinated persons underscored the need to vaccinate complement-deficient persons against meningococcal disease.

Among individuals with LCCD, C5-deficient patients and persons who receive C5 inhibitors such as eculizumab that is approved for in the treatment of conditions such as paroxysmal nocturnal hemoglobinuria[250] and has also been administered to select individuals with aHUS[251] or severe Shiga-toxin-associated HUS,[252] are unique because they do not have the capacity to generate C5a, a key anaphylatoxin and chemoattractant. Through its action on the C5a receptor (C5aR), C5a upregulates FcγR and activates neutrophils, which is critical for opsonophagocytic killing of meningococci. Whole blood from a C5-deficient patient failed to kill a group B isolate of *Nm* even in the presence of high titers of specific antibody that otherwise supported killing when pure C5 was added back.[253] Studies are needed to determine the efficacy of meningococcal vaccines in the subset of individuals who are C5-deficient (either inherited or secondary to pharmacologic inhibition).

In other complement-deficient states, the benefit of vaccinating individuals is less clear. Fijen et al. reported the responses of 53 diverse complement-deficient individuals (7 C3-deficient, 19 properdin-deficient, and 27 terminal component-deficient) to immunization with the tetravalent capsular polysaccharide vaccine.[254] All patients generated functional antibody responses that were comparable to that seen in normal individuals and revaccination of two C3-deficient and 17 late complement-deficient patients resulted in robust increases in antibody titers.[255] Although not statistically different, the rates of disease in the 8 y following the initial vaccination were lower than in the 8 y preceding immunization. Other studies have reported sub-optimal antibody responses in C1-, C2-, C4-, and C3-deficient humans and animal models[256-263] and as such, it may be worth documenting antibody responses to immunization in these instances. Finally, no data that have adequately evaluated the efficacy of immunization in C3-deficient persons; presumably these individuals rely on clearance of antibody-coated bacteria by engagement of Fc receptors as bacteria traverse sinusoids of the reticuloendothelial system.

Concluding Remarks

While the past decade has witnessed considerable progress in our understanding of various aspects of meningococcal pathogenesis, in large part because of advances in sequencing technologies, there are still several gaps in our knowledge of how this bacterium escapes immune surveillance and resides often for long periods as a harmless a commensal and why in some instances it causes catastrophic invasive infection. Development of humanized mice will likely facilitate studies of meningococcal pathogenesis in vivo. Antibodies and complement undoubtedly play major roles in determining clinical outcomes following acquisition of a strain, but the role of phagocytes and other aspects of cell-mediated immunity merit further research. We are only beginning to appreciate the layers of complexity of meningococcal complement evasion mechanisms. Elucidating how meningococci can evade the complement system is of particular importance as newer meningococcal vaccines are developed. As an example, elucidation of how fHbp-negative invasive isolates[264] evade complement is germane in light of fHbp-based vaccines being licensed for clinical use. Studies of the efficacy of newer conjugate vaccines and protein vaccines in complement-deficient individuals merit consideration. Finally, clinicians should be aware of the high risk of meningococcal disease in complement-deficient individuals, especially as clinical indications for therapeutic inhibitors of complement are increasing and newer complement inhibitors are being developed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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