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Biology and pathogenesis of the evolutionarily successful, obligate human bacterium *Neisseria meningitidis*

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Abstract

For at least two hundred years, *Neisseria meningitidis* (the meningococcus), the cause of epidemic meningitis and sepsis, has inflicted rapid death, disability and fear on disparate human populations. The meningococcus is also recognized as a highly successful commensal organism exclusively found in humans. The evolution of *N. meningitidis* to an exclusive human commensal and to a sometimes fulminant and fatal pathogen represents an important case study in microbial pathogenesis. We review the general status of our knowledge of pathogenesis of meningococcal carriage, transmission and virulence behavior with particular emphasis on the relevance of research on this topic to vaccine development.

Keywords

Neisseria meningitidis; pathogenesis; meningitis

1. Introduction

Beginning with the initial clinical descriptions of distinct outbreaks in Geneva in 1805 [1] and New Bedford, Massachusetts in 1806 [2] and its isolation in 1887, the meningococcus has been recognized to cause endemic cases, case clusters, epidemics and pandemics of meningitis, devastating septicemia, and less commonly, pneumonia, septic arthritis, pericarditis, chronic bacteremia and conjunctivitis in hundreds of thousands of individuals worldwide each year [3]. Mortality remains at ~10% or higher in developing countries and morbidity includes limb loss, hearing loss, cognitive dysfunction, visual impairment, educational difficulties, developmental delays, motor nerve deficits, seizure disorders and behavioral problems [4]. Curiously, no reports of the very distinct outbreaks of epidemic meningococcemia and meningitis occur prior to 1806 or reports of epidemics in the meningitis belt of subSaharan Africa prior to ~1900.

Humans are the only host for the meningococcus. *N. meningitidis* is a frequent member of human pharynx and upper respiratory flora with reports of the organism at the buccal mucosa, rectum, urethra, urogenital track and in dental plaque. The most common natural habitat of the meningococcus is the human epithelial of the human naso- and posterior pharynx. *N. meningitidis* is carried in the pharynx by 8–25% of the human population, translating to hundreds of millions of carriers worldwide with adolescents being a principal reservoir [3].

1.1 *Neisseria meningitidis*

N. meningitidis is a Gram-negative *B*-proteobacterium. Marchiafava and Celli (1884) described intracellular oval micrococci in a sample of cerebrospinal fluid (CSF) and Anton Weichselbaum (1887) isolated the organism, distinguished pneumococci from meningococci in CSF, and designated the latter as *Diplococcus intracellularis meningitidis* [5,6]. The sometimes intracellular location of *N. meningitidis* in human cells is an important feature of meningococcal pathogenesis.

Invasive strains of meningococci express capsules and meningococcal strains were originally distinguished by differences in capsular polysaccharide structure designated serogroups [7]. Virulence determinants include the polysaccharide capsule, outer membrane proteins including pili, the porins (PorA and B), the adhesion molecule, Opc, iron sequestration mechanisms and endotoxin (lipooligosaccharide) [4]. *N. meningitidis* is now classified into 13 serogroups based on the immunogenicity and structure of the polysaccharide capsule. Further classification into serosubtype, serotype and immunotype is based on class 1 outer membrane proteins (PorA), class 2 or 3 (PorB) outer membrane proteins and lipopoly[oligo]saccharide structure, respectively [3,4]. PorA is an important target for bactericidal antibodies. In addition to these specific virulence factors, *N. meningitidis* has evolved genetic mechanisms that result in high frequency phase and antigenic variation and molecular mimicry. Capsule switching, due to allelic exchange of capsule biosynthesis genes by transformation, is one example that can allow the meningococcus to evade immune detection [8].

A genetic typing system based upon polymorphisms in multiple housekeeping genes (Multilocus Sequence Typing, MLST) is now the gold standard for molecular typing and has defined hyper-virulent meningococcal lineages [9]. Why hypervirulent meningococcal lineages are more pathogenic has been a subject of considerable interest. Based on sequencing of eight genomes, the chromosome is between 2.0 and 2.1 megabases in size and contains about 2000 genes [10–12]. Each new strain sequenced has identified 40–50 new genes and the meningococcus shares about 90% homology at the nucleotide level with either *N. gonorrhoeae* or *N. lactamica.* Mobile genetic elements including IS elements and prophage sequences make up ~10% of the genome [11]. Other than the capsule locus, no core pathogenome has been identified suggesting that virulence may be clonal group dependent. Given that transformation is an efficient mechanism of genetic exchange and that meningococci have acquired DNA from commensal *Neisseria* spp. and other bacteria (e.g. *Haemophilus*) as well as phages, the gene pool for adaptation and evolution is quite large. Genome plasticity and phenotype diversity through gain and loss of DNA or, for example, through DNA repeats, is a characteristic of meningococcal evolution. This is in contrast to the relatively conserved genomes of for example *Bacillus anthracis.* The acquisition of the capsule locus by horizontal transfer possibly from *Pasteurella multocida* or *P. hemolytica* [12] appears to be a major event in the evolution of the pathogenicity of the meningococcus.

1.2 Pathogenesis and epidemiology

Although rates of sporadic disease can reach ~5–10/100,000 population [1], a key characteristic of meningococcal disease are epidemic outbreaks. Seasonal epidemics (usually due to serogroup A) occur yearly in subSaharan Africa and cyclical pandemics have occurred there every 8–10 years for the last 100 years. During seasonal epidemics and cyclical pandemics the incidence can climb to >1/1000 population for weeks before the frequency of disease declines in the immediate outbreak area. For example, despite partially effective vaccines, in the dry seasons in Burkina Faso of the subSaharan African meningitis belt over 5,000 cases of meningitis per week (rate of >680 cases/100,000 population) are reported, an almost yearly occurrence in the country [13]. In developing countries, meningococcal epidemics cause catastrophic expense, which contributes to the cycle of poverty and hence the disorganization

of social structures. Serogroups B, C and Y are associated with sporadic disease, case clusters and outbreaks seen in the United States, Canada, New Zealand, South America, Europe and other parts of the world [3]. Serogroup W-135 is responsible for recent worldwide outbreaks associated with pilgrims returning from the Hajj [14]. The different characteristics of outbreaks are caused by hypervirulent lineages as defined by MLST. The worldwide W-135 outbreaks were caused by W-135 strains of the ST-37 clonal complex most often associated with serogroup C disease and outbreaks. The introduction of new virulent clones into a population can change the epidemiology and the clinical spectrum of meningococcal disease. As an example, the emergence of serogroup Y meningococci in the United States in the late 1990's has been associated with increased rates of meningococcal disease in all ages including bacteremic pneumonia in older adults and elderly. The recent emergence of serogroup X meningococci in Niger [15] highlights the need for continued surveillance for new clonal complexes.

Meningococcal disease has the highest incidence in infants and children aged <4 years and adolescents [4]. The early stages of disease can mimic viral infections such as influenza, but the disease course may be fulminant. Thus, it can be difficult to identify and treat the disease quickly. Rapid progression of the disease from bacteremia and/or meningitis to life-threatening septic shock syndrome or meningitis can occur within the first few hours after initial symptoms appear. Because of these parameters, prevention through vaccination is the best option for the control of this disease in a community. While significant progress is being made in understanding meningococcal pathogenesis and in new meningococcal vaccines and vaccine strategies, challenges remain. Dissecting the molecular basis of meningococcal pathogenesis also has important scientific lessons for understanding bacterial emergence, pathogenic genome structure, horizontal genetic exchange, and innate and adaptive immune responses.

2. Meningococcal colonization and carriage

Colonization of the upper respiratory mucosal surfaces by *N. meningitidis* is the first step in establishment of a human carrier state and invasive meningococcal disease. Meningococcal transmission among humans occurs largely through respiratory droplets and secretions, but the inoculum size needed for transmission is unknown. Acquisition of *N. meningitidis* in the upper respiratory tract may be asymptomatic or may infrequently result in local inflammation, invasion of mucosal surfaces, access to the bloodstream and fulminant sepsis or focal infections such as meningitis [4]. Meningococcal disease usually occurs 1–14 days after acquisition of the pathogen [3]. Acquisition may also result in upper respiratory and pharyngeal meningococcal carriage. The duration of carriage can vary from days to months.

2.1 Meningococcal adhesion and cell invasion

Adhesion to human mucosal surfaces is essential for meningococcal survival. Not suprisingly, multiple *N. meningitidis* adhesins recognizing different human host receptors have been identified. Adhesin redundancy is a hallmark of the meningocccus. Recognized adhesins include Pili, PilC, PilQ, Opa, Opc, LOS, Factor H binding protein, Por A, HrpA, PorB and NadA. Proposed or demonstrated receptors include platelet activating factor, CD46, CEACAM1, vitronectin and alpha-actinin integrins, Complement receptor 3, laminin and the GP96 scavenger receptor [16].

Stephens *et al.* [17] first developed a human nasopharyngeal organ culture model and found that meningococci specifically bind nonciliated columnar epithelial cells and induce pseudopodia that ultimately results in internalization of meningococci within these cells (Figure 1). Initial contact of meningococci with nasopharyngeal epithelial cells is mediated by Type IV pili, the receptor for which may be the I-domain of integrin alpha chains or possibly CD46 [18,19]. Meningococci proceed to proliferate on the surface of human nonciliated

epithelial cells, forming small microcolonies at the site of initial attachment. Capsule blocks close adhesins other than pili, and thus may aid meningococcal transmission) from mucosal surfaces. Attachment may activate two-component regulatory systems, leading to loss or downregulation of capsule. Close adherence of meningococci to the host epithelial cells results in the formation of cortical plaques and leads to the recruitment of factors ultimately responsible for the formation and extension of epithelial cell pseudopodia that engulf the meningococcus [18]. Intimate association is mediated by the bacterial opacity proteins, Opa and Opc with CD66/CEACAMs and integrins, respectively, on the surface of the epithelial cell and is one trigger of meningococcal internalization [20]. However, other bacterial-epithelial cell associations, including the meningococcal adhesin NadA and meningococcal LOS, have less defined roles in this process [18]. The formation of the cortical plaque cell membrane protrusions (Figure 1) stems from the organization of specific molecular complexes involving the molecular linkers ezrin and moesin (known as ERM [ezrin-radixin-moesin] proteins), along with the clustering of several membrane-integral proteins, including CD44, intracellular adhesion molecule (ICAM) 1, and cortical actin polymerization [21,22].

Cortical actin polymerization requires phosphorylated cortactin, which leads to membrane protrusion and pseudopodial formation and internalization of *N. meningitidis*. Some meningococcal LOS mutants are poorly invasive, show structurally altered actin polymerization and are defective in the recruitment and phosphorylation of cortactin [21]. The next steps of meningococcal internalization, intracellular survival and transcytosis through the basolateral tissues and dissemination into the bloodstream [4,17] are less well studied. Intracellular meningococci reside within a membranous vacuole and are capable of translocating through the epithelial layers within 18–40 hrs. Meningococcal intracellular survival is determined by factors including IgA_1 protease, which degrades lysosome-associated membrane proteins (LAMPs), thus preventing phagosomal maturation and upregulation of expression of capsule. Meningococci are capable of intracellular replication and this is due in part to the capacity of the organism to acquire iron through specialized transport systems, such as the hemoglobin binding receptor (HmbR), transferring binding protein (TbpAB) and lactoferrin binding protein (LbpAB) [23].

2.2 Meningococcal carriage

The powerful contributions of conjugate vaccines against a variety of encapsulated bacteria achieved through the induction of herd immunity, including serogroup C meningococci, provides a compelling rationale for an improved understanding of the biology of the commensal behavior of meningococci. From an evolutionary perspective, the interactions of meningococci and the human nasopharynx are key. Meningococcal carriage and transmission, not disease, determine the global variation and composition of the natural population of meningococci.

Meningococcal carriage and its consequences are a dynamic model. Cross-sectional carriage studies sample only those bacteria that can be swabbed at a given time and may lack sensitivity. Carriage studies may be difficult to interpret if intracellular or submucosal tissue sites within the nasopharynx are important. It is difficult to understand why meningococci have evolved multiple mechanisms of cell invasiveness (e.g. Opa, NadA) if this capability is not contributing to fitness. The biological role of capsular polysaccharide in carriage/transmission is not well understood. Capsule-deficient strains may be transmitted efficiently, so the theory of resistance to dessication during transit or capsule antiadhesive properties promoting loss does not seem strong. Capsule is not required for efficient carriage. The adaptive advantage of switching between the capsulate and non-capsulate state seems likely to provide a fitness advantage possibly for close adherence and initial steps in cell invasion. There is evidence of differences in propensity for carriage associated with the different capsular polysaccharides (serogroups), e.g. low carriage of serogroup C meningococci.

As noted, the redundancy of adhesins possessed by meningococci, e.g., pilus, Opa, NadA and their striking allelic variation, is impressive. The source of this variation is strongly influenced by lateral transfer of genetic information (recombination) and meningococci are naturally transformable. Nevertheless, most individuals are colonized with only one meningococcal strain (at least based on carriage studies) and this must place some constraints on the opportunity for genetic exchange between heterologous strains. Meningococcal adhesins are not known to be characteristic of other commensal *Neisseria* although this is not well studied. Thus, there appears to be a "knowledge gap" on how allelic meningococcal diversity occurs with such frequency. Can the observed diversity be adequately explained by strong selection of rare events, do we have an inadequate knowledge of carriage of multiple distinct strains of Nm as a consequence of the limitations of current carriage studies or is there a larger genetic pool available to meningococci in the nasopharynx?

The relationship between meningococcal carriage rates and meningococcal disease and even the use of carriage prevalence as a proxy for predicting outbreaks of meningococcal disease has received considerable study. The important measure in terms of disease is the rate of acquisition of meningococci of hypervirulent lineages, not overall meningococcal carriage. The probability of meningococcal disease after the acquisition of *N. meningitidis* declines very sharply, such that invasive disease becomes unlikely 10–14 days after acquisition. Too little research has been done on the interactions of N. *meningitidis* with other upper respiratory tract commensals/pathogens. For example, carriage of pneumococci appears to be a risk factor for meningococcal carriage [24].

3. Invasive meningococcal disease

Meningococci can cross mucosal surfaces, enter the bloodstream and produce a systemic infection. Once access to the bloodstream is obtained, meningococci may multiply rapidly to high levels. Meningococci may also translocate across the blood-meningeal barrier, proliferate in the CNS and cause meningitis. The ability to cause invasive disease depends on environmental factors, meningococcal virulence factors and lack of a "protective immune response." Environmental factors that impair the integrity of the human nasopharyngeal mucosa such as tobacco [4], exposure to low humidity, dust and co-infections [25,26] increase the incidence of invasive meningococcal disease.

Meningococci may penetrate between epithelial or endothelial cells, transcytoses through them or are carried across the epithelial and endothelial barriers within cells (Trojan horse theory). There is epidemiologic, biological and pathological evidence for each of these events. The molecular events noted *in vitro* for epithelial cell invasion (see section 2.1) are similar with endothelial cells. The role of "permeability factors" is not well understood. For example, the strong association of the "dry season" and the winds of the Hamantan with epidemics in sub-Saharan Africa may be related to damage to nasopharyngeal mucosal barriers.

Major meningococcal contributors to the invasive meningococcal disease include: capsular polysaccharide, other surface structures [pili, OMPs (e.g. PorA, PorB, Opa, Opc), lipooligosaccharide (LOS)] and genotype. Resistance to complement-mediated lysis and phagocytosis is determined by the expression of the capsule and lipooligosaccharide [27]. Meningococcal endotoxin released in blebs also plays a major role in the inflammatory events of meningococcemia and meningococcal meningitis [4].

LOS plays a role in the adherence of the meningococcus [28] and activation of the innate immune system. Severity of meningococcal sepsis has been correlated with circulating levels of meningococcal LOS [4]. Pili and other OMPs, facilitate the adherence of the meningococcus to endothelial surfaces. While multiple meningococcal invasion virulence factors are influence

invasive disease and some are the focus of new vaccines [29], two structures LOS and capsule play critical roles in meningococcal virulence.

3.1 Capsular polysaccharide

Capsular polysaccharide (CPS) plays a crucial role both in invasive meningococcal disease. Capsule, composed of sialic acid derivatives (with the exception of serogroup A), provides the organism with antiphagocytic properties enhancing its survival in the bloodstream or in the central nervous system (CNS). Meningococci that do not express capsular polysaccharides rarely, if ever, cause invasive disease. Capsule enables the meningococcus to evade complement-mediated and phagocytic killing and is the basis for immunological serogrouping. Thirteen structurally distinct capsular serogroups have been described, six of which cause the majority of invasive disease (A, B, C, Y, X, and W-135) [4,30].

Of the six major invasive meningococcal serogroups, four (B, C, Y and W-135) express capsules containing sialic acid. Serogroups B and C are homopolymers of N-acetylneuraminic acid in a α2→8 (B) or α2→9 (C) linkage, respectively [30]. Serogroups Y and W135 are composed of alternating disaccharide repeat units of sialic acid and D-glucose or D-galactose, respectively. Serogroup A is composed of (α1→6)-linked N-acetylmannosamine-1-phosphate, while serogroup X expresses (α1→4) linked N-acetyl-D-glucosamine 1-phosphate. The genetic organization of the meningococcal capsule polysaccharide synthesis (*cps*) locus in all strains expressing capsules is comprised of three regions within a 24 kb virulence island of low G+C content [31]. Region A (*synA-D*) encodes proteins involved in sialic acid synthesis and elongation of the sialic acid polymer. The first three genes (*synA-C*) of this region are highly homologous among sialic acid capsule-expressing serogroups, while the gene encoding the capsule polymerase (*synD*) is serogroup specific [32]. The CPS biosynthesis proteins are believed to form an assembly complex at the cytoplasmic membrane. Regions B (*ctrE* and *ctrF*) and C (*ctrA-D*) encode proteins involved in the capsule membrane transport protein complex and are required for export of capsule polymers through the inner and outer bacterial membranes. CtrD-catalyzed ATP hydrolysis powers the transport of the CPS across the cytoplasmic membrane through a channel formed by the transmembrane domains of the integral membrane protein, CtrC. In order to transfer CPS from the cytoplasmic membrane to the outer surface of the cell, a link between the inner and outer membranes is formed by the membrane fusion protein, CtrB, so that the substrate is transported through the designated outer membrane porin, CtrA. Region B contains two genes, *ctrE* (*lip*A) and *ctrF* (*lipB*), which are also required for export of lipidated polymer to the outer membrane of the meningococcus [30].

3.2 Meningococcal endotoxin

Meningococcal endotoxin, lipopolysaccharide (LPS) or lipooligosaccharide (LOS) is composed of a lipid A and a core oligosaccharide. Repeating “O-antigen” polysaccharide characteristic of enteric Gram-negative bacteria are not found in many important respiratory Gram-negative bacteria such as *N. meningitidis*, *Bordetella* or *Haemophilus* spp., and the term LOS has been used [33].

The core oligosaccharide of meningococcal LPS contains an inner core structure of two heptoses and an *N*-acetyl-glucosamine linked by Kdo_2 to the lipid A. The heptose residues provide linkage to the oligosaccharide residues of the α-, β- and γ-chains. Meningococcal lipid A is responsible for much of the biological activity and toxicity of meningococcal endotoxin. However, Kdo-lipid A is the minimal structure molecule resulting in maximal biological activity [35]. On an equi-molar basis (molecules of *N*-acetyl glucosamine), wild-type meningococcal endotoxin stimulates greater MyD88-dependent and –independent cytokine release than other endotoxins [34]. The symmetrical acylation of the lipid A molecule, physico-

chemical properties associated with the structure and extensive lipid A phosphorylation may explain the unique biologic properties of meningococcal endotoxin.

Meningococcal lipid A is composed of a disaccharide of pyranasol N-acetyl glucosamine residues. Each glucosamine sugar is acylated at the 2-position with N-linked β hydromyristic acid (3-O (12:0)-C14:0), with lauric acid (C12:0) residues attached to the β-hydroxy group of 3-OH C14:0 fatty acids. Additionally, the 3-position of each glucosamine is acylated with O-linked β-hydroxylauric acid (3-OH C12:0). The 1 and 4′ positions of the glucosamine residues typically following lipid A synthesis contain mono phosphate groups glycosidically linked at the 1 position and another phosphate group linked at the 4′ position of the β-glucosamine (the basal 1-, 4′a -di or bis phosphorylated species). However, the phosphorylation of meningococcal lipid A and the addition of phosphoethalanomine groups is quite extensive and variable.

N. meningitidis expresses multiple LPS structures. Early studies, grouped these structures into 12 immunotypes (L1–12), of which nine have now been assigned chemically defined structures. Genotyping studies of the immunotype strains by Jennings et al. [36] indicate that LOS expression falls into two broad categories: those strains that have a phase variable lacto-neotetraose (LNT) α-chain with fixed PE decorations to the inner core, and those strains expressing a phase locked LNT α-chain and variable additions of PE and glucose to the inner core. Phase variation of the expression of the α-chain resulted in shorter structures termed L1 and L8. Immunotyping screens of panels of invasive disease and carriage isolates has indicated that carriage isolates commonly expressed L1 and L8, while invasive isolates were characterized by the expression of LOS with long α-chains. The long LNT chain LOS structures mimic the I and i human antigens. Discrimination of LOS with different decorations on the inner core by immunotyping has proven to be difficult due to cross reactivity with common epitopes in the α-chains. However, Scholten *et al*. [37] indicated that approximately two-thirds of invasive disease isolates expressed L3 immunotypes while the remainder expressed L2,4.

N. meningitidis strain MC58, which has been used to analyze the interactions of meningococci with host cells, expresses a phase variable LNT and a fixed inner core containing O-3 PE attached to Heptose II (HepII). Partial sialylation of the LNT by the LOS sialyltransferase Lst, results in the mixed expression of L3 (sialylated) and L7 (unsialylated) versions of this structure by strain MC58. Phase variable expression of the LOS glycosyltransferase, *lgtE*, results in the expression of L8, an unsialylated short α-chain consisting of Glc-Gal. Virji et al. [38] showed that MC58 expressing L8 had higher adherence to immortalized endothelial cell lines than the L3 expressing strain. From this model, it was deduced that the long α-chain may interfere with Opc mediated attachment and invasion, either directly through binding the sialylated LOS or indirectly by sterically shielding the surface exposed loops of Opc. However, Lambotin et al. [22] has suggested that intact long α-chain LOS may have a direct role in triggering invasion as this structure is required to promote a phosphoinositide-3-kinase (P13K)/Rac 1 co-stimulatory signal required for cortical plaque formation.

LPS structure influences pathogenesis. One example is the evolution of resistance of meningococci to cationic antimicrobial peptides (CAMPs). CAMPs are constitutively present in macrophages and neutrophils and inducibly produced by epithelial cells at mucosal surfaces, play an important role in host defense against microbial infection and are key components of the innate immune responses. In phagocytes they are constitutively present and stored in the cytoplasmic granules and are released into the developing phagolysosome. In contrast, non-phagocytic cells reacting to stimuli (including endotoxin) can inducibly synthesize CAMPs. Owing to their non-oxidative killing action, CAMPs are thought to perform an important role in innate host defense against invading pathogens. In addition to their antibacterial activities they can act as signaling and/or chemotactic molecules that connect innate and adaptive

immune responses. *N. meningitidis* is intrinsically highly resistant to CAMPs, such as polymyxin B (PxB) (MIC ≥512 μg) [39]. Genes encoding the proteins responsible for the biosynthesis and attachment of Ara4N are absent in the meningococcal genomes. Both lipid A modification by LptA, adding phosphoethanolamine to the lipid A headgroup, and the MtrC-MtrD-MtrE efflux pump plays important roles in meningococcal resistance to PxB CAMPs [41].

The TLR4 receptor is critical to the innate immune responses to bacterial endotoxins including meningococcal LPS [40]. Activation of TLR4 by endotoxin requires association with the accessory protein MD-2, an *N*-glycosylated [43] 19–27 kilo-Dalton protein that is expressed in both a soluble and a membrane bound form. Binding of endotoxin LPS or LOS to MD-2 in association with TLR4 can lead to dimerization or oligomerization of two or more TLR4 receptors and subsequent cellular activation. MD-2 belongs to a family of proteins which express an ML (MD-2-related lipid-recognition) domain [41–46]. The protein contains 160 amino acids which form a “clamshell” binding site for hydrophobic ligands inserted between two-pleated sheets [48,49]. Related proteins include MD-1 associated with RP105 on B cells; the dust mite antigens, Der p2 and Der f2; Niemann-Pick disease C2 (NPC2) ; and the GM2-activating protein important in Tay-Sachs gangliosidosis. Der p2 exhibits the most homology to MD-2, and its NMR-determined solution structure includes a β-folded binding pocket for an unidentified lipid ligand. MD-2 directly interacts with the lipid A of meningococcal endotoxin. Several important crystal structures have been published recently (human MD-2 bound to the human TLR4 antagonist, lipid IVa [47]; the human TLR4-MD-2 complex associated with the endotoxin antagonist eritoran [48]; and mouse MD-2 complexed to mouse TLR4. These data provide an opportunity to explore the precise interaction between MD-2 and meningococcal endotoxin.

3.3 Co-factors of invasive meningococcal disease

Known risk factors for invasive meningococcal disease include smoking, crowding, deficiencies in terminal complement components, asplenia, travel to epidemic areas [34], and deficiencies in mannose binding lectin [49]. Meningococcal disease can affect persons of all age groups, but higher rates of invasive disease in developed countries are seen in infants and children less than 4 years-old, adolescents, military recruits and groups were crowding and new exposures occur such as college students living in dormitories [3].

Antibodies, complement and phagocytes are major host defense mechanisms necessary in the protection against invasive meningococcal disease. The meningococcal carrier state constitutes a natural immunizing process with appearance of antibodies in serum two weeks after nasopharyngeal colonization of pathogenic and non pathogenic meningococci. Goldschneider *et al*. [50] found that age specific incidence of meningococcal disease is inversely proportional to the prevalence of serum bactericidal antibodies (SBA) to the meningococcus during the first 12 years of life. Sixty to 80% of young adults will have detectable meningococcal SBA. Further, sera from susceptible hosts lack antibodies to disease causing strains of meningococcus during an epidemic. Finally, individuals with congenital or acquired deficiencies of immunoglobulins are at increased risk for invasive meningococcal disease [51–53]. Although specific immunity is generally protective, this immunity is not absolute; meningococcemia can occur in individuals with preexisting antibody titers that are considered protective.

“Experiments of nature” indicate the important role of complement deficiency and polymorphisms of other innate host determinants, such as the FcII receptor, as risk factors for meningococcal disease. Older hypotheses, such as the role of blocking antibodies (IgA) proposed many years ago, have not been advanced and may even have been forgotten by current researchers. The lack of satisfactory animal models is a frustrating limitation to the study of meningococcal pathogenesis.

Complement is required for both meningococcal bactericidal activity and for opsonophagocytosis [54]. Individuals deficient in both the early and late components of the complement system are at increased risk for meningococcal disease [51–53]. Compared to the general population, these patients usually experience less severe, more recurrent disease at an older age with less common serogroups. Ten to 20% of invasive meningococcal disease in adults is associated with a complement defect. The mannose–binding lectin (MBL) pathway of complement activation can be genetically variable and is associated with difference in susceptibility to meningococcal disease in one third of all cases [4,49]. Other genetic polymorphisms affecting the risk of acquiring meningococcal disease have also been described (TNF, FcγRIIA, FcγRIII, PAI-1, ACE-1, IL-1Ra, IL-1β, TLR4) [4].

There is limited knowledge on the co-factors influencing the spread and severity of meningococcal disease. Low absolute humidity may damage the nasopharyngeal mucosa allowing meningococci to pass the mucosal barrier or be transmitted by coughing. The dry season and sub-Saharan outbreaks is noted above. In countries with a temperate climate susceptibility to meningococcal disease is highest in the winter when absolute humidity is low. There is also evidence that viral (influenza) and mycoplasma respiratory infections can predispose to meningococcal disease, perhaps by damaging mucosal surfaces, altering dynamics of adherence, colonization and spread and impairing mucosal immunity. The role of mucosal immunity in prevention and exacerbation of meningococcal disease requires more analysis in the future.

The association of HIV and meningococcal disease has not been well studied. Population based studies in the US indicate an increased risk (~7 fold) [55], but this has not been noted in African outbreaks. However, the high prevalence of HIV in African and Asian countries may influence the carriage or the susceptibility to and severity of meningococcal disease, as demonstrated earlier for pneumococcal infections.

4. Meningococcal pathogenesis and vaccines

Capsule has been the target antigen for meningococcal vaccines. Meningococci causing disease usually express one of six capsular serogroups (A, B, C, W-135, X and Y) and successful polysaccharide and polysaccharide-protein conjugate vaccines based upon four of these polysaccharides are now available [4]. Serogroup C conjugate vaccines have impacted the incidence of serogroup C disease in industrialized countries [56] where the conjugate vaccines have been introduced, but the large serogroup A epidemics in the developing world remain. In addition, there is no current licensed vaccine for serogroup B, as this capsular serogroup mimics human antigens and is a poor immunogen [4]. Serogroup B also causes prolonged outbreaks such as those seen over the last two decades in the US Pacific Northwest (Oregon, parts of Washington State), Brazil, Norway and New Zealand [57]. Outer membrane vesicles (OMV), combinations of outer membrane proteins with or without LOS are predicted, based on serum bactericidal assays, to confer protection against serogroup B disease [29,57]. Meningococcal LOS-based vaccines are under development and show promise. Unraveling the pathogenic mechanisms of this devastating, evolutionarily successful and obligate human pathogen has significance for new meningococcal vaccine development, the understanding of human sepsis, the design of sepsis antagonists and vaccine adjuvants.

The implementation of meningococcal vaccination programs can have a tremendous influence on the epidemiology of meningococcal disease. The example of the MenC conjugate vaccines indicates that vaccination influences not only meningococcal disease, but also nasopharyngeal transmission and carriage of meningococci. This effect has markedly increased the effectiveness (doubled) of Men C vaccination programs. However as noted for *H. influenzae* b conjugate vaccines, conjugates may have different immunologic properties that influence

herd immunity. Conjugate vaccines against other serogroups (ACYW135) are currently in use or under development. Carriage studies before and after implementation of vaccination programs are urgently needed to identify the effect of vaccination on meningococcal carriage and disease.

Replacement of *N. meningitidis* in the context of vaccine selection pressure exerted by conjugate vaccines has received considerable attention. Although no evidence for an increase in non-vaccine serogroups had been observed to date (for example, the UK experience with MenC conjugate vaccines), this phenomenon, well documented for pneumococcal conjugate vaccines [58], needs to be monitored prospectively in other epidemiological settings and for the other (A,W,Y) conjugate vaccines.

MLST has been shown to be currently the best method for global typing of meningococci and in understanding the alterations of carriage and disease before and after vaccination. However, the degree of robustness of MLST in predicting the antigenic profile needs further analysis. Especially in the context of new protein-based meningococcal vaccines the expansion of current typing protocols needs consideration. MLST uses conserved core sequences and the problem is that it does not necessarily predict associations between many vaccine antigens because of the lack of concordance introduced by recombination.

Surveillance systems especially in developing countries for meningococcal disease clearly need to be enhanced. As has been the case for the W135 epidemic in Africa, surveillance may direct the preparation of vaccines against new clonal strains or specific serogroups [4]. PCR or other non culture tests may be especially useful in diagnosis in regions where patients frequently receive antibiotics before reaching hospitals. Current WHO reports may underestimate the real disease burden, because bacteremia and severe (fatal) disease are often not reported. Further underestimation may be due to limited resources for establishing a diagnosis. In many, but not all developing countries at least one laboratory is available for the surveillance of meningococcal disease but limitations in the availability of diagnostic and typing methods may further result in underestimation of disease burden. Accurate surveillance remains an important component before and after the introduction of new meningococcal vaccines. Consideration of clinical manifestations other than septicemia and meningitis will provide information on the total meningococcal disease burden and increase the cost-effectiveness of new meningococcal vaccination programs.

5. Disclosure of potential conflict of interest

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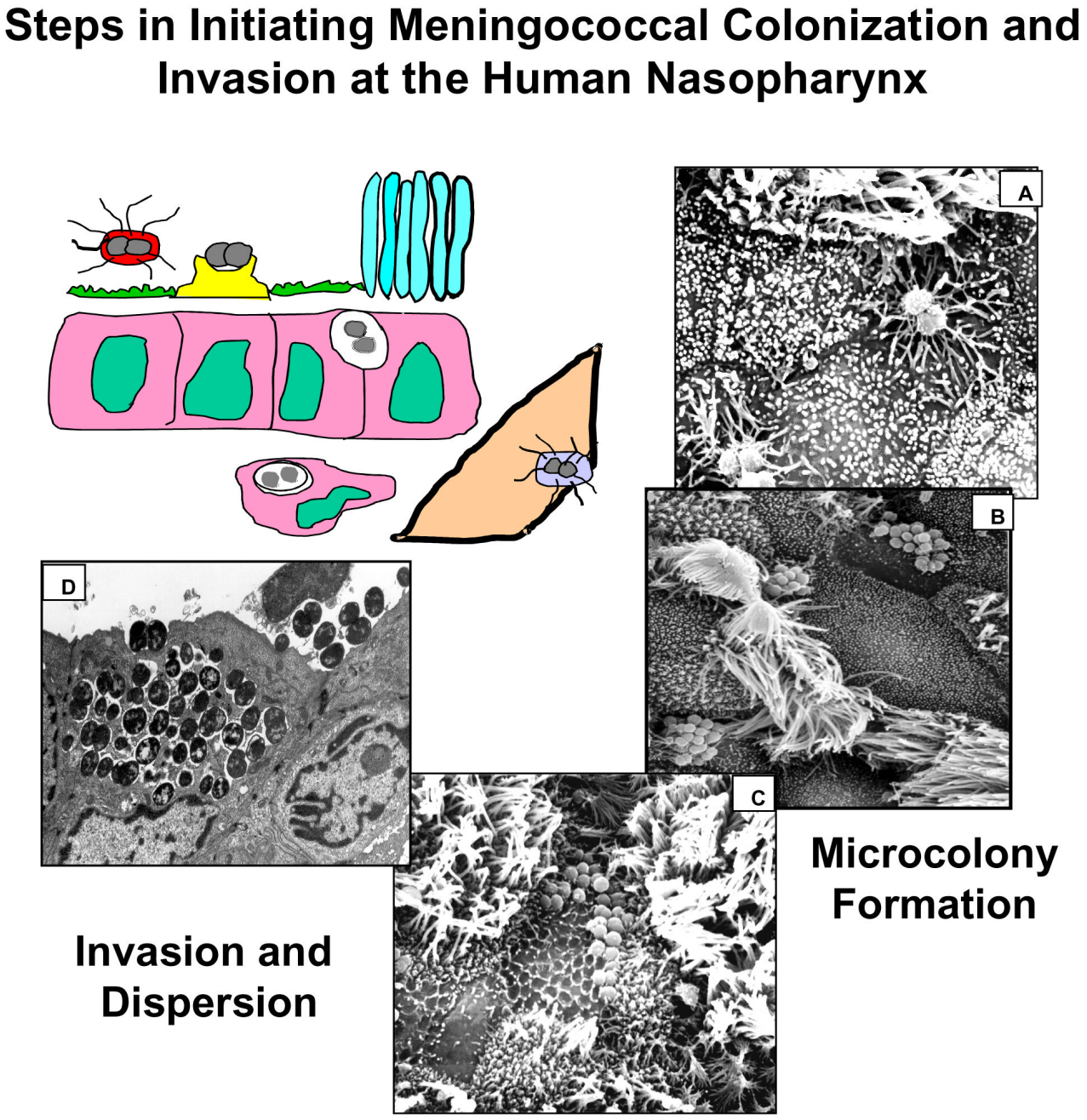

Figure 1.
A. Adhesion and introduction of cell microvilli. **B.** Microcolony formation. **C.** Cortical plaque formation and close adherence. **D.** Human epithelial cell invasion.