Neisseria meningitidis: pathogenesis and immunity
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The recent advances in cellular microbiology, genomics, and immunology has opened new horizons in the understanding of meningococcal pathogenesis and in the definition of new prophylactic intervention. It is now clear that Neisseria meningitidis has evolved a number of surface structures to mediate interaction with host cells and a number of mechanisms to subvert the immune system and escape complement-mediated killing. In this review we report the more recent findings on meningococcal adhesion and on the bacteria-complement interaction highlighting the redundancy of these mechanisms. An effective vaccine against meningococcal B, based on multiple antigens with different function, has been recently licensed. The antibodies induced by the 4CMenB vaccine could mediate bacterial killing by activating directly the classical complement pathway or, indirectly, by preventing binding of fH on the bacterial surface and interfering with colonization.

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Introduction
Meningococcal meningitis and sepsis are devastating diseases which still affect people with an incidence varying between 0.5 and 1000 cases/100 000 depending on the epidemiological areas. The etiological agent is Neisseria Meningitidis, a gram negative diplococcus and an obligate human pathogen. Meningococcus colonizes the nasopharyngeal mucosa and is most typically carried asymptomatically by approximately 10% of the population at any given time [1]. Rates of asymptomatic carriage increase dramatically in certain conditions and at different ages, with a peak in the adolescence, especially when people get together such as at college and military entry. While colonization of the nasopharynx is a common event, the disease is rare but can cause death or permanent disability in as little as 24 hours from the first recognizable symptoms [2].

To be able to colonize, survive in the bloodstream and spread, the bacterium might use different strategies to evade the immune system and to adapt to different environments. Capsule is the main virulence factor and its expression undergoes genetic regulation during pathogenesis. Cell adhesion and biofilm formation are inhibited by capsule and therefore the expression needs to be downregulated or lost during carriage. On the other hand, capsule is important for survival in the blood and is consequently upregulated during invasion into the bloodstream [2]. Twelve different meningococcal serogroups have been defined based on the capsular polysaccharides of which five: A, B, C, W135, and Y are responsible for the majority of the disease, and with serogroup X more recently identified as cause of sepsis and meningitis in Africa [3]. The capsular polysaccharide represents the major antigen and, in the case of A, C, W135, and Y is the basis for the conjugate vaccines, shown to be very effective in preventing the disease in all age groups [2]. Serogroup B capsular polysaccharide is poorly immunogenic because it is identical to the polysialic acid present in many human glycoproteins including the neural cell adhesion molecule and as such cannot be used as vaccine antigen [4]. The design of a broad protein-based vaccine against meningococcal B had met numerous challenges. The first protein based MenB vaccine contained outer membrane vesicles (OMVs) and was shown to be efficacious against the OMV-derived strain. OMV protective ability resides mainly in the PorA antigen, an integral membrane protein with β-barrel structure and protruding loops made by variable sequences which constitute the main epitopes and which are the driving cause for the OMV vaccine strain specificity [5].

This antigenic diversity of the meningococcal surface proteins has been the main limitation in the design of broadly protective meningococcal vaccines. The challenge of the antigen diversity has been addressed by two different approaches: one based on multiple antigens identified by ‘reverse vaccinology’ which resulted in the development of the first MenB vaccine licensed so far in Europe, Australia, Canada and Chile (4CMenB) [6,7]; the other based on the combination of two variants of the same antigen and which is in late stage of development [8].

Here we will review the most recent findings in meningococcal pathogenesis and on the mechanisms of immune escape, with particular emphasis on the biological activity...
of the antigenic components of the newly developed MenB vaccines.

**Meningococcal colonization**

Adhesion to mucosal surface is an essential step in the establishment of a carrier state. Acquisition of meningococcus may be asymptomatic but in some circumstances may result in local inflammation, invasion of mucosal surfaces with access to the blood-stream which may result in fulminant septicemia and/or meningeal inflammation. The first step in meningococcal colonization is adhesion to epithelial cells of the nasopharynx and is mediated by Type IV pili, which are also involved in many stages of the infection process, such as adhesion to endothelial cells, bacterial aggregation, twitching motility, bacterial migration and natural transformation [9]. They are multimeric proteins, with pilin E as the scaffold of the pilus which spans the inner and the outer membranes and extrudes through a pore formed by PilQ [10]. Over 20 proteins are required for a correctly assembled and functional Type IV pilus [11]. PilE and PilV are involved in adhesion to host cells and recently shown to activate β2-adrenergic receptor (β2-AR), promoting the endothelial signaling events enabling *Neisseria* translocation through the brain endothelium [12,13]. The mechanism of Type IV pil adhesion has been further elucidated by Bernard et al. who identified CD147, a member of the immunoglobulin (Ig) superfamily, as receptor for PilE and PilV-mediated adhesion to human brain or peripheral endothelial cells and shown the role of CD147 in vascular colonization by meningococci [14*]. However, the role and the localization of some of the pil components is still under discussion. Imhaus et al. recently demonstrated that, despite the assigned function for pilX, as mediating bacterial aggregation and for pilV as an adhesion, the two pilus components are localized in the periplasm and very interestingly, they exert their activity indirectly by changing the number of Type IV pil per bacterium and as consequence, regulating competence, aggregation and host-cell interaction [15*].

Intimate adhesion is then mediated by the opacity proteins, Opa, and Opc, with a typical integral membrane protein structure, which bind to carcinoembryonic antigen cell adhesion molecule (CEACAMs) receptor and extracellular matrix components, respectively [16]. Although Pili, Opc, and Opa represent the most important and the most studied adhesins of meningococcus, additional antigens have been described as having a role in adhesion, such as NhA (*Neisseria hia/hsf* homologue), a trimeric autotransporter with homology to Hia and Hsf adhesins of *Haemophilus influenzae* which binds to extracellular matrix proteins, heparan sulfate and laminin and facilitates attachment to host epithelial cells [17,18], App (Adhesion and penetration protein, homologous to *Haemophilus* Hap) [19,20] and NadA (*Neisseria* adhesin A), a trimeric autotransporter belonging to the Oca family (oligomeric coiled-coil adhesins) [21]. NadA expression levels vary among isolates and is upregulated by niche-specific signals via the transcriptional regulator NadR [22], which binds the *nadA* promoter and represses transcription. DNA-binding activity of NadR is attenuated by 4-hydroxyphenylacetic acid (4-HPA), a natural molecule released in human saliva, thus leading to the de-repression of *nadA* in *vivo* [22]. NadA forms stable trimers on the bacterial surface which mediate binding to epithelial cells through interaction with a protein receptor molecule differentially expressed by various epithelial cell lines [23]. NadA interacts with Hsp90 (Heat shock protein 90), and this influences its adhesive phenotype [24]. The three dimensional structure reveals a novel TAA (trimeric autotransporter adhesins) organization made mostly of a coiled-coil with protruding wing-like structures forming a head-like domain [25].

The presence in meningococcus of multiple adhesins (Figure 1) with different receptor specificities suggests that they could interact cooperatively with different receptors on the same target cells, or may act at different stages during infection, mediating *Neisseria* adhesion to different cell types at different sites.

Although many aspects of the *Neisseria* pathogenesis have been elucidated, and sophisticated *in vitro* and *in vivo* systems are now available (tissue culture bilayers, human nasopharynx transplantation, mice transgenic for human receptors) to further explore the role of these proteins in virulence and their potential as target for therapeutic intervention, to be a promising vaccine candidate, the antigen must induce complement-mediated bactericidal antibodies, the established correlate of protection in humans. In this respect, NadA is the most promising vaccine candidate among the adhesins identified so far, being able to induce high level of bactericidal antibodies in humans and a component of the 4CMenB vaccine.

**Figure 1**

Schematic illustration of *Neisseria meningitidis* outer membrane proteins involved in colonization, App (adhesion and penetration protein); Type IV pili; Opc (opacity protein C); NhA (*Neisseria* hia/hsf homologue); NadA (*Neisseria* adhesin A).
Neisseria interaction with complement components

Antibodies are key in protecting against invasive meningoococcal infection. Goldshneider et al. demonstrated that age disease incidence was inversely proportional to the prevalence of bactericidal antibodies in the sera [26]. The complement system plays a major role in innate immune defense against meningococcal disease and subjects with terminal complement deficiencies are more susceptible to invasive meningococcal disease [27]. There are three main complement pathways: the classical pathway (CP) which is mediated by the binding of the C1 component to the antigen–antibody complex; the lectin pathway which recognizes specific microbial patterns composed of saccharides and related molecules and the alternative pathway (AP) which is mediated by the binding to C3 [28].

The three pathways involve the cleavage of C3, the central component of the complement cascade, which in turn induces the formation of the membrane attack complex (MAC) and forming a pore in the membrane of the target cell, induces cell death [28].

Factor H binding protein

In the last few years many proteins have been identified as having a role in complement evasion. The most studied is fhbp (factor H binding protein), also known as GNA1870 [29] or RLPO086 [30], which interacts with human Factor H, an inhibitor of the alternative complement pathway [31,32]. Fhbp is one of the antigenic components of the licensed 4CMenB vaccine [7*], or the sole component of a vaccine under clinical development [8*]. The binding of fhbp to Factor H enhances meningococcal serum resistance allowing the bacterium to grow in the blood. The structure of fhbp or of fhbp-fH (domains 6 and 7) complex have been solved [33**] and the residues responsible for this interaction have been identified and mutagenized to generate new non-fH binding with enhanced immunogenicity in a fH transgenic mouse model [34–37]. Interestingly, the side chains of fhbp that interact with fH resemble the glycosaminoglycan binding region of fH on host cells. Therefore Neisseria is able, through fhbp, to recruit factor H by mimicking the host [33**].

Redundant complement escape mechanisms

More recently, a number of other surface-exposed antigens able to inhibit the alternative complement pathway have been identified, suggesting that the activity of fhbp is not unique and that meningococcus possesses a variety of mechanisms that enable it to evade the human immune system. Additional bacterial components able to bind factor H and inhibit the alternative complement pathway are: Nspa (Neisseria surface protein A), which, like fhbp, binds domains 6 and 7 of human fH, and acts cooperatively with fhbp [38,39*]; PorB2 (Porin B2) which binds not only human fH but also regulates the alternative complement pathway of baby rabbit and infant rat [40]; sialylated lipooligosaccharide (LOS) which enhances the binding of fH C-terminal domains 18–20 to bacteria when C3 fragments are deposited on the bacterial surface [39*]. Moreover, Neisserial heparin binding antigen (NHBA), an important component of the 4CMenB vaccine, is able to bind heparin in vitro through an Arg-rich region and this property correlates with increased survival of Neisseria in human blood [41]. Heparin is known to interact with many complement components such as factor H, C4b-binding protein and vitronectin. Therefore the establishment of the NHBA–heparin complex on the meningococcal cell surface could recruit complement inhibitors, and in turn prevent complement activation.

Capsular polysaccharides also modulate multiple pathways of the complement cascade. Serogroup Y and W135 enhance activation of the AP by enhancing C3 activation and deposition. Moreover, Agarwal et al. recently demonstrated that B, C, W, and Y capsular polysaccharide inhibits the CP by inducing less C4b deposition, limiting the ability of antibodies to mediate bacterial killing. In this study, the different strains showed different susceptibility to anti-fHbp antibodies and complement, despite similar antibody binding to the bacterial surface, suggesting possible implications for the activity of fHbp-based vaccines [42]. PorA has also been shown to bind C4b-binding protein (C4bp), the major CP inhibitor. PorA-expressing strains were more resistant to complement mediated lysis in a serum bactericidal assay, suggesting that binding to C4bp helps N. meningitidis to escape CP complement activation [43].

An additional mechanism to evade the complement system is mediated by NaIP, a serine protease autotransporter [44] which cleaves C3 four amino acids upstream from the natural C5 cleavage site and produces shorter C3a-like and longer C3b-like fragments. The C3b-like fragment is degraded, resulting in lower deposition of C3b on the bacterial surface which is then reflected in higher meningococcal serum resistance [45].

Moreover, genes encoding for fhbp, LOS sialylation and capsule biosynthesis are regulated by increase in temperature, suggesting that this signal triggers meningococcal immunoevasen and complement resistance [46].

The evidence that N. meningitidis uses different mechanisms to subvert the complement system (Figure 2) and to survive and multiply in the bloodstream, and the redundancy in complement evasion mechanisms may have important implications on meningococcal vaccines based solely on one antigen.
Future outlook
The evaluation of new meningococcal vaccines presents a number of challenges. Studies on the evolution of meningococcal pathogenesis, on the molecular epidemiology and on the impact on complement evasion mechanisms will be of primary importance during implementation of such vaccines.

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References and recommended reading
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• of special interest
•• of outstanding interest


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An important study showing the role of pili and pilX in regulating the number of Type IV pil per bacterium and influencing competence, aggregation and host-cell interaction.


An important study showing the 3D structure of the FH-fhbp complex, and the strategy adopted by meningococci to recruit factor H by mimicking the host.


Study showing the relative roles of fhbp, NspA and LOS in recruiting complement factors and regulating the alternative pathway.


