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REVIEW ARTICLE

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Interactions between encapsulated *Neisseria meningitidis* and host cells

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Summary A major feature of *Neisseria meningitidis* is its ability to invade human brain meninges. To access the meninges, the bacteria must cross the blood-brain barrier (BBB), which is one of the tightest barriers in the body. Therefore, *N. meningitidis* must have evolved some type of sophisticated means to bypass the physical properties of this cellular barrier. As *N. meningitidis* is encapsulated when present in the bloodstream, this review will focus on the mechanisms that encapsulated *N. meningitidis* has developed to interact with host cells and will suggest ways in which these mechanisms may be helpful for crossing the BBB.

Key words *Neisseria meningitidis* · Blood–brain barrier · Adhesion · Pili · Microbial pathogenesis

Introduction

Neisseria meningitidis is an extracellular pathogen responsible for meningitis and septicemia in humans. *N. meningitidis* colonizes the nasopharynx and spreads aerially from person to person. In a small percentage of people colonized with *N. meningitidis*, the meningococci gain entry to the bloodstream, where they may cause meningococcemia and/or progress to the cerebrospinal fluid (CSF). After crossing the blood-brain barrier (BBB), the microorganism finally causes meningitis. To reach the meninges from the throat, the meningococcus must interact with two cellular barriers, one in the nasopharynx and the other, the BBB, in the brain. The BBB consists of two structures. The first consists of the endothelium of the brain capillaries, in which the endothelial cells differ from those in peripheral capillaries by the presence of tight junctions limiting paracellular flux. The second is the choroid plexus, located in the ventricles, the major site of CSF synthesis. In the choroid plexus, endothelial cells are fenestrated, and the BBB is formed by tight junctions at the ventricular surface of the epithelial cells. Meningococci need two kinds of virulence factor to invade the meninges after colonizing the throat: (i) attributes responsible for bloodstream survival and dissemination, and (ii) components mediating the meningococcal interaction with cellular membranes, which facilitates bloodstream invasion and the crossing of the BBB. One of the virulence factors responsible for bloodstream survival and dissemination is the polysaccharide capsule. Its importance has been demonstrated by the observation that unencapsulated isolates are usually found in the nasopharynx, whereas bacteria recovered from the blood or the CSF are encapsulated. Virulence factors mediating interactions with cells include several bacterial components but the sequence of events in the interaction is not clear. This article reviews recent developments in studies of the bacterial attributes used by virulent meningococci to interact with human cells. The article also suggests mechanisms by which the meningococcus-cell interaction may lead to the crossing of a cellular barrier such as the BBB.

Type IV pili

Type IV pili are filamentous structures consisting of protein subunits that extend from the bacterial surface (Fig. 1). They are of paramount importance to the pathogenic process, as shown by the fact that primary cultures of clinical isolates of pathogenic *Neisseria* are invariably piliated. In vitro, their role in promoting adhesion to epithelial and endothelial cells is essential and has been well established. In encapsulated meningococci, pili seem to be the main feature involved in the initiation of the meningococcus–host cell interaction; the extent of adhesion of non-piliated encapsulated bacteria to mammalian cells is limited [11]. Only a few encapsulated meningococci are internalized, with most remaining as extracellular adherent pathogens. The production of pili is also associated with other phenotypic characteristics such as a high level of competence for transformation by exogenous DNA, bacterial auto-agglutination, and twitching motility. Pilus biogenesis and the description of the pilus-associated proteins responsible for pilus-mediated adhesion will be reviewed extensively elsewhere. The current model suggests that PilC molecules (110 kDa) are located in pilus fibers and carry a cell-binding domain [16]. PilC molecules are therefore thought to be the adhesins responsible for pilus-mediated adhesion. Various strains of meningococcus have one or two *pilC* loci. However, in case in

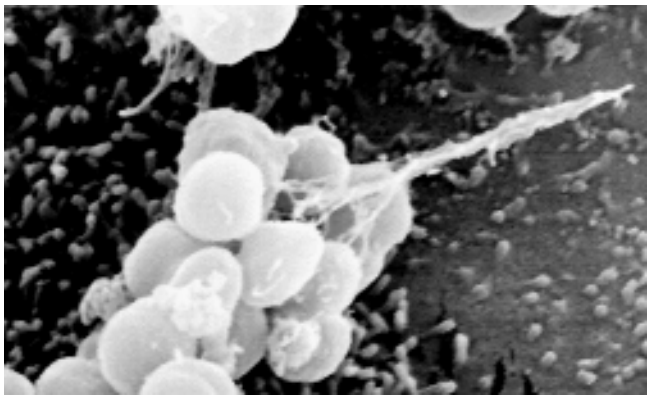

Fig. 1 PilT-meningococcus expressing pili and interacting with T84 epithelial cells. Pili can be seen as mediating this interaction

which two PilC proteins can be produced, only one, designated PilC1, is adhesive, whereas the other (PilC2) is unable to promote pilus-mediated adhesion [11]. The reason for this difference is unknown. It may be due to the lack of a cell-binding domain or to the inability of non-adhesive PilC2 proteins to migrate in the pili. Extensive comparison of the primary sequences of various PilC proteins does not help to explain this phenomenon. The transcription of the *pilC1* adhesive allele in the meningococcus is under tight regulation. Initial interactions between meningococci and their host cells upregulate the transcription of *pilC1*, but not of *pilC2*. This upregulation is essential to obtain a fully adhesive phenotype [20], demonstrating that the interaction of meningococci with cells leads to crosstalk between the bacteria and the cells.

Pilin is the pilus subunit. It is believed to be incapable of interacting directly with eukaryotic cells, and instead, to play an essential role as the fiber scaffold. However, some pilin variants are more efficient than others in enhancing bacterial cell interactions, due to their ability to favor agglutination of pili, which then form large bundles [8, 10]. Two mechanisms may account for the increase in adhesiveness due to bundled pili: (i) bundled pili increase bacteria–bacteria interactions, and (ii) bundled pili may strengthen the interaction of the adhesin with its eukaryotic receptor, because several adhesin molecules are present at the extremity of a bundle. Although the mechanism by which some pilins induce pilus aggregation is not known, comparison of the primary sequences of weakly and strongly adhesive derivatives suggests that localized charge modifications may play a role in the bundling process [12].

A major feature of pathogenic *Neisseria* pilins is that they are glycosylated. A covalently bound, *O*-linked, *N*-acetyl glucosamine-α1,3-galactose (GlcNAc-α1,3-Gal) was first identified in gonococcus pilin [12] and then in the pilin of one meningococcus strain [9]. The sugar identified in another meningococcus strain is a trisaccharide, a digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose [18]. Glycosylation is not required for pilus biogenesis; indeed, nonglycosylated pilins result in the formation of pili that tend to aggregate more and to form bundles larger than those obtained with glycosylated pilins. Consistent with this, more soluble truncated monomers of pilin are produced by strains with glycosylated pilin than by strains in which pilin is not glycosylated [9]. The fact that glycosylation increases the amount of soluble pilin monomers suggests that unassembled pilin molecules may have a function. A cell-binding domain is present in the constant region of the pilin monomer [8]. Based on pilin structure, this site would not be accessible in the pilus fiber. Soluble monomers of pilin could signal to cells via this cell-binding domain and have an effect independent of their role as the building block of pili. Other posttranslational modifications have been reported for pilin: a glycerophosphate [19] and a phosphorylcholine epitope are linked to pilin. The role of these modifications has not been explored. Recently a phosphate has been shown to be linked to Ser 68. This phosphate promotes straighter and/or less bundled pili by charge modification [3].

The complement regulatory protein (CD46) has recently been recognized as a pilus receptor for pathogenic *Neisseria* [6]. Piliated *Neisseria* are unable to bind cells of nonhuman origin such as CHO cells; they are capable, however, of binding to CHO cells expressing human CD46. The attachment of bacteria is blocked by monoclonal antibodies against CD46 and by recombinant CD46 protein produced in *Escherichia coli*. The pilus-associated molecule responsible for this attachment has not been identified, but the adhesive forms of PilC are the best candidates. The consequences of this initial attachment of pili to CD46 are unknown, but this interaction is believed to send a signal to the host cells. However, it does not result in the efficient uptake of bacteria into the cells.

Other bacterial components

Class 5 (Opa) and Opc proteins The Opa-associated (Class 5) proteins are basic outer membrane proteins with a molecular weight of ~28 kDa. They form a family of proteins, each encoded by a different *opa* gene. Opa proteins are subject to phase variation due to the occurrence of reversible sequence variation in the 5´ coding region of their structural genes. Opc proteins are outer membrane proteins of similar size and physico-chemical properties to Opa proteins. However, the 2 groups are structurally different and have only limited sequence similarity. Opc also undergoes phase variation, due to transcriptional regulation [17]. The level of transcription of *opc* may vary from zero through intermediate to high. The expression of Opc protein is restricted to a subset of meningococcus strains.

Opc and Class 5 proteins mediate interaction between unencapsulated meningococci and eukaryotic cells. In encapsulated bacteria, neither Opa nor Opc has any effect on bacterial interactions with host cells. Class 5- and Opc-mediated cellular interactions are also strongly inhibited by lipooligosaccharide sialylation [2, 23, 24].

Opa proteins facilitate the interaction of meningococci with epithelial cells, polymorphonuclear leukocytes and, to a lesser extent, endothelial cells. It has been established that Opa proteins bind to members of the CD66 family, mediating adhesion to and invasion of epithelial cells [25, 26]. In interactions with polymorphonuclear leukocytes, binding to CD66 leads to the phagocytic elimination of Opa-producing meningococci. Opc significantly increases the adhesion of bacteria and the extent of invasion into Chang epithelial cells and human umbilical vein endothelial cells [23], but only if Opc is present at high levels [17]. Opc causes the adhesion of non-piliated meningococci and promotes the interaction of unencapsulated variants, not only with human cells, but also, to a lesser degree, with cells from other mammals. Cell-surface proteoglycans of cultured epithelial cells have recently been shown to be the receptors for Opc [2]. However, the exact nature of the proteoglycan receptors recognized by these adhesins/invasins has not been elucidated. Opc has also been reported to facilitate the adhesion to and invasion of endothelial cells via the binding of vitronectin in a trimolecular complex. Opc-producing meningococci interact with vitronectin, which they use to attach to integrin $\alpha_v\beta_3$ on the apical surface of the endothelial cells [25]. In strains lacking Opc, the binding of the meningococcus to proteoglycan may be mediated by some other component, such as an Opa protein.

Other outer membrane components Several other bacterial components regulate bacteria–cell interactions. Sialylation of lipooligosaccharide negatively regulates Opa and/or Opc mediated interactions [2, 22]. Similar observations have been made for the meningococcus capsule [11].

Neisserial porins have been shown to translocate spontaneously as functional voltage-gated ion channels into the plasma membranes of eukaryotic cells, causing a transient change in membrane potential and interfering with cell signaling [21]. Although the role of porins as promoters of bacterial entry into the host cell has not been addressed in meningococcus, it has been shown that the PorA and PorB proteins in meningococci are capable of nucleating actin [4]. This suggests that the translocated *Neisseria*-encoded porins are involved in host cell actin reorganization during infection.

IgA1 protease, which is believed to be crucial for mucosal colonization, has recently been shown to have a significant role in intracellular survival. It cleaves the LAMP1 protein, thereby preventing phagolysosomal fusion. Thus, IgA1 protease must be involved in the survival of pathogenic *Neisseria* inside epithelial cells [1, 7].

Meningococcal diseases

A major biological feature of the meningococcus is its ability to colonize the nasopharynx asymptomatically. The mechanism of colonization is not known, and neither is the mechanism by which meningococci cross the mucosal barrier. A possible way is via the M cells present at the tonsillar sites. Transcytosis through these cells might be an efficient route of invasion for meningococcus, as has been described for enteric pathogens [5].

Another interaction between the meningococcus and a cellular barrier takes place when it crosses the BBB. As stated above, at this stage the meningococci are encapsulated and pili seem to be the only bacterial factor that can mediate any cellular interaction. The BBB consists of epithelial and/or endothelial cells that have tight junctions that limit paracellular flux. Recent in vivo data have demonstrated both that the meningococcus interacts with these structures and that the movement across these monolayers requires pilus-mediated adhesion and more specifically, correlates with the presence of an adhesive form of PilC [13]. A better understanding of all the steps involved in the movement of the meningococcus across the BBB requires the development of in vitro models. As the main cellular characteristic of the BBB is the existence of tight junctions that limit paracellular flux, the in vitro model for studying this step should be a monolayer of endothelial cells (tight-junction forming) and/or epithelial cells. Although considerable progress has been made, BBB models using human brain endothelial cells are not yet available. This has led to the use of epithelial monolayers of polarized human cells with organized tight junctions similar to those present in the BBB. Using such a model, which only reflects some aspects of the movement of the meningococcus across the BBB, the bacterium has been shown to cross a monolayer by crossing the cells without destroying the intercellular junctions [14]. Although bacteria have not been seen between the cells, this does not exclude the possibility that this route is used. Initially, the adhesion of the meningococcus is localized, resulting in the formation of clumps of bacteria on the apical surface of the monolayer. The bacteria then spread onto the surface of the cells as the clumps disappear and are replaced by a monolayer of meningococcus covering the cells, producing a diffuse adhesion phenotype. At this stage, bacteria adhere intimately and firmly to the apical membrane and, in some places, they are found intracellularly.

Knowledge about the bacterial factors involved in all these steps is very poor. As stated above, pilus-mediated adhesion is likely to be most critical during the first step of localized adhesion. Surprisingly, by the diffuse adhesion stage, the meningococci have lost their pili [15]. There are several possible explanations for this. Firstly, crosstalk between bacteria and cells could lead to the downregulation of genes involved in pilus biogenesis. Secondly, pili could be retracted, thereby bringing the outer membrane of the bacterium and the cell plasma membrane into contact. The loss of piliation following the localized adhesion step suggests that pili are not involved per se in the intimate attachment observed during diffuse adhesion. The best candidates for this function would be the Opa/Opc proteins. However, two observations are not consistent with these molecules being involved in this intimate attachment; (i) *opc*$^-$ isolates are also

capable of intimate attachment [14], (ii) a naturally occurring Opa⁻ variant is also able to undergo intimate attachment [14]. The role of porins in this process is unknown. Nonpiliated bacteria cannot achieve diffuse adhesion, which suggests that the initial phase of localized adhesion is necessary for the production of unidentified factors that lead to the diffuse adhesion phenotype. Furthermore, there is evidence that PilT, a cytoplasmic nucleotide-binding protein involved in pilus retraction, is required to disperse bacteria, causing a change from the localized to the diffuse adhesion pattern, and to induce the loss of piliation and intimate attachment [15]. Therefore, pili may be seen as sensory organs which, after recognizing their receptor on the surface of the cells, transduce a signal to the bacteria, which in turn upregulate the expression of unidentified components necessary for intimate attachment and for the signaling events linked to this step. The loss of piliation at the diffuse adhesion step could result from the same signaling event. The cellular mechanisms driving the transcytosis of bacteria have yet to be explored.

Identification of the additional factors responsible for the specificity of the bacterial pathogenesis will be facilitated by the availability of the sequence of the meningococcal genome and its comparison with that of *Neisseria gonorrhoeae*.

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