MicroReview

Interactions of pathogenic *Neisseria* with host cells. Is it possible to assemble the puzzle?

Xavier Nassif,* Céline Pujol, Philippe Morand and Emmanuel Eugène

INSERM U411, Faculté de Médecine Necker-Enfants Malades, Université René Descartes, Paris, France.

Summary

*Neisseria meningitidis* and *Neisseria gonorrhoeae* are human pathogens that have to interact with mucosa and/or cellular barriers for their life cycles to progress. Even though they both give rise to dramatically different diseases, the use of *in vitro* models has shown that most of the mechanisms mediating cellular interactions are common to *N. meningitidis* and *N. gonorrhoeae*. This suggests that bacterial cell interactions may be essential not only for pathogenesis but also for other aspects of the bacterial life cycle that are common to both *N. meningitidis* and *N. gonorrhoeae*. This manuscript will review the most recent developments concerning the mechanisms mediating cellular interaction of pathogenic *Neisseria* and will then try to put them into the perspective of pathogenesis and bacterial life cycle.

Introduction

*Neisseria meningitidis* (MC) and *Neisseria gonorrhoeae* (GC) are two human extracellular pathogens that have to interact with cellular barriers for their pathogenesis. *Neisseria meningitidis* colonizes the nasopharynx and eventually spreads into the bloodstream before crossing the blood–brain barrier to induce meningitis. *Neisseria gonorrhoeae* colonizes and invades the epithelium of the genitourinary tract leading to a localized inflammatory process that is rarely followed by bacteraemia. For these pathogens, which infect only humans and do not survive in the environment, the ability to be pathogenic is a minor aspect of their life cycle. It is likely that a large part of their genome is devoted to coding for bacterial attributes necessary for colonization and survival in their niche, which is the nasopharynx for *N. meningitidis* and the genitourinary tract of asymptomatic carriers for *N. gonorrhoeae*.

The use of *in vitro* models has allowed much progress to be made for identifying bacterial components and cellular receptors involved in these interactions. Most of these are common to both pathogenic *Neisseria*. This is consistent with the fact that *N. meningitidis* and *N. gonorrhoeae* belong to the same genospecies (Guibourdenche et al., 1986) and share many common genetic, biochemical and antigenic features. However, the role of these factors *in vivo* is mainly unknown. The goal of this review is to emphasize recent findings concerning molecular aspects of the interactions between pathogenic *Neisseria* and host cells, and then to address the possible role these attributes could have in the life cycle of these pathogens.

Type IV pili

Type IV pili are filamentous structures emanating from the bacterial surface composed of protein subunits. They are of paramount importance to the pathogenic process, as revealed 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 the case of encapsulated meningococci, pili seem to be the main attribute involved in the initiation of MC cell interaction, and the adhesion of non-piliated encapsulated bacteria with mammalian cells remain limited (Nassif et al., 1994). Furthermore, after pilus-mediated interaction of encapsulated MC, few bacteria are internalized, and most of them remain as extracellular adherent pathogens. Production of pili is also associated with a number of other phenotypes, such as high level competence for transformation by exogenous DNA, bacterial autoagglutination and twitching motility. Pili biosynthesis as well as the description of the pilus-associated proteins responsible for pilus-mediated adhesion will be reviewed extensively in other articles in this series. The current model suggests that 110 kDa PilC molecules are located in pilus fibres and carry a cell-binding domain (Rudel et al., 1995). PilC molecules are therefore viewed as adhesins responsible for pilus-mediated adhesion. In
GC, two pilC alleles have been identified both of which are adhesive. In contrast, different strains of MC have one or two pilC loci. However, when two PilC proteins can be produced, only one, designated PilC1, is adhesive; the other (PilC2) is unable to promote pilus-mediated adhesion (Nassif et al., 1994). The reason for this discrepancy is unknown, and could be because of either the lack of a cell-binding domain or that these non-adhesive PilC2 proteins are unable to migrate in the pili. Extensive comparison of the primary sequences of various PilC proteins does not help to explain this phenomenon. Transcription of the pilC1 adhesive allele in MC is under tight regulation. Initial interactions of MC with host cells upregulate transcription of pilC1 but not pilC2. This upregulation is essential for obtaining a full adhesive phenotype (Taha et al., 1998), demonstrating that the interaction of MC with cells leads to a cross-talk between the bacteria and the cells.

The pilin is the pilus subunit. It is believed to be incapable of interacting directly with eukaryotic cells but rather to play an essential role as the fibre scaffold. However, some pilin variants are more efficient than others in enhancing bacterial cell interactions, owing to the ability of these variants to favour agglutination of pili, which then form large bundles (Marceau et al., 1995; Marceau and Nassif, 1999). Two mechanisms may explain the enhancement of adhesiveness by bundled pili: (i) aggregative pili increase bacteria–bacteria interactions and (ii) bundled pili could reinforce the interactions of the adhesin with its eukaryotic receptor because several adhesin molecules are present at the extremity of a bundle. The mechanism by which some pilins induce pilus aggregation is not known, but comparison of the primary sequence of low and high adhesive derivatives suggests that localized charge modifications might play a role in the bundling process (Parge et al., 1995).

A major feature of pathogenic Neisseria pilins is that they are glycosylated. An O-linked galactose, alpha-1,3 GlcNAc, was first identified in GC pilin (Parge et al., 1995) and then in the pilin of one MC strain (Marceau et al., 1998). In another MC strain, the sugar identified is a trisaccharide, a digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose (Stimson et al., 1995). Glycosylation is not required for pilus biogenesis, and in fact non-glycosylated pilins are responsible for the formation of pili that tend to aggregate more and to form larger bundles than those obtained with glycosylated pilins. Consistent with this is the larger proportion of soluble, truncated monomers of pilin produced by strains having a glycosylated pilin than that observed when pilin is non-glycosylated (Marceau et al., 1998). The fact that glycosylation increases the amount of soluble pilin monomers suggests that unassembled pilin molecules could have a function. A cell-binding domain is present in the constant region of the pilin monomer (Marceau et al., 1995). According to the pilin structure, this site would not be accessible in the pilus fibre. One may speculate that 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 post-translational modifications have been reported for pilin: a glycerophosphate (Stimson et al., 1996) and a phosphorylcholine epitope are linked to pilin (Weiser et al., 1998). The role of these modifications has not yet 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 (Forest et al., 1999).

The complement regulatory protein (CD46) has recently been recognized as a pilus receptor for pathogenic Neisseria (Källström and Jonsson, 1998). Piliated Neisseria are unable to bind cells of non-human origin, such as CHO cells; however, they are capable of binding to CHO cells expressing human CD46. 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 between pili and CD46 remain to be assessed, but this interaction is believed to send a signal to the host cells; however, it is not efficient at causing the internalization of the bacteria inside the cells. The discovery that CD46 is a pilus receptor will lead to a better understanding of the cellular events after pilus-mediated interactions and of the exact role of pilus-mediated adhesion in the pathogenesis of both MC and GC.

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 are expressed in most MC and all GC and are structurally similar in both species. They form a family of proteins, each being encoded by a distinct opa gene. Opa proteins are subject to phase variation because of the occurrence of reversible sequence variation in the 5’ coding region of their structural genes. A single gonococcal strain may carry up to 12 opa genes, whereas MC strains have fewer loci (three or four).

Opc proteins are outer membrane proteins that have similar size and physicochemical properties as the Opa proteins. However, they are structurally distinct and have only limited sequence similarity. Opc also undergoes phase variation; this process is due to transcriptional regulation (Sarkari et al., 1994). The transcription of opc may vary from zero to the intermediate or high level. Even though an opc-like gene has recently been described in GC, the expression of an Opc protein is restricted to a subset of MC strains.
The role of Opa proteins as mediating cellular interactions has been extensively studied in GC. Much of the more recent studies were performed using non-piliated derivatives of strain MS11, producing different Opa proteins. The MS11 Opa repertoire and the corresponding nomenclature is shown in Table 1. In this strain, the Opa proteins can be divided in two groups according to the type of molecules they recognize on the surface of the mammalian cells. The first group is represented by OpaA (Opa50), which binds to a heparan sulphate proteoglycan of the syndecan family (van Putten and Paul, 1995), thus resulting in adhesion and internalization of gonococci by some epithelial cell lines such as Chang, Hec1B, HeLa and ME180. This entry process resembles phagocytosis rather than macropinocytosis and involves a signalling pathway requiring stimulation of the phosphatidylcholine specific phospholipase and acidic shingomyelinase (Grassmé et al., 1997). In some cell lines such as CHO, Hep-2 and HeLa, this proteoglycan-mediated adherence is not sufficient to promote entry of OpaA-expressing gonococci. An additional interaction between OpaA and a serum protein is required for invasion (Duensing and van Putten, 1997; Gomez-Duarte et al., 1997; van Putten et al., 1998a). This interaction involves fibronectin for Hep2 and HeLa cells, and vitronectin for CHO cells. Fibronectin or vitronectin bind specifically to OpaA proteins and to the corresponding integrin via the RGD sequence, thus bridging the gonococci to the host cells. In this case, the entry process results from a concerted action of the heparan sulphate proteoglycan receptors and the integrin receptors. Protein kinase C (PKC) inhibitors block vitronectin-mediated invasion, suggesting that this latter internalization pathway possibly involves PKC (Dehio et al., 1998). Like OpaA, OpaC also has heparan sulphate-binding properties (Chen et al., 1997).

The second group of Opa proteins encompasses the other 10 alleles that mediate interaction with eukaryotic cells via CD66 (Chen et al., 1997; Gray-Owen et al., 1997a). The CD66 family includes four different forms: the CD66a (biliary glycoprotein; BGP), CD66b (CEA gene family member 6; CGM6), CD66c (non-specific cross-reactive antigen; NCA), and CD66d (CEA gene family member 1; CGM1). CD66b is not bound by any of the Opa proteins, whereas the other members of this family are differentially recognized by the Opa proteins (see Table 1) (Bos et al., 1997; Gray-Owen et al., 1997b). Recognition does not involve the carbohydrate moiety of CD66, and the CD66 receptor specificity exhibited by neisserial Opa variants is controlled by determinants in the CD66 N-domain (Bos et al., 1998). The CD66 molecules are produced differentially by polymorphonuclear, epithelial and endothelial cells. Initially, Opa proteins, which do not bind to surface proteoglycans, were identified by their ability to produce an interaction with polymorphonuclear leucocytes (PMN), thus causing opsonin-independent uptake by professional phagocytes (Fischer and Rest, 1988). This CD66-mediated phagocytosis requires a Src-like tyrosine kinase- and Rac1-dependent signalling pathway (Hauck et al., 1998). The different specificities of Opa proteins for CD66 receptor

### Table 1. The MS11 Opa proteins repertoire.

<table>
<thead>
<tr>
<th>Opa proteins&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>rOpa&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Molecules recognized&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>OpaA</td>
<td>Opa50</td>
<td>Heparan sulphate proteoglycan</td>
<td>Invasion of epithelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitronectin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>OpaB</td>
<td>Opa57</td>
<td>CD66a, CD66c, CD66d, CD66e</td>
<td>Uptake by professional phagocytes in an opsonin-independent pathway</td>
</tr>
<tr>
<td>OpaC&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Opa52</td>
<td>CD66a, CD66c, CD66d, CD66e</td>
<td>Transcytosis across a tight-junction-forming monolayer of epithelial cells</td>
</tr>
<tr>
<td>OpaD</td>
<td>Opa56</td>
<td>CD66a, CD66e</td>
<td>Increase adhesion to TNF-α-activated HUVECs</td>
</tr>
<tr>
<td>OpaE</td>
<td>Opa57</td>
<td>CD66a, CD66e</td>
<td></td>
</tr>
<tr>
<td>OpaF</td>
<td>Opa58</td>
<td>CD66a, CD66c, CD66d, CD66e</td>
<td></td>
</tr>
<tr>
<td>OpaI</td>
<td>Opa59</td>
<td>CD66a, CD66e</td>
<td></td>
</tr>
<tr>
<td>OpaJ</td>
<td>Opa60</td>
<td>CD66a, CD66c, CD66d, CD66e</td>
<td></td>
</tr>
<tr>
<td>OpaK</td>
<td>Opa61</td>
<td>CD66a, CD66e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Opa63</td>
<td>CD66a, CD66e (+/- -)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> This comparison is similar to the one published by Bos et al. (1997).

<sup>b</sup> Designation of naturally occurring Opa variants by Swanson et al. (1988).

<sup>c</sup> Designation of recombinant Opa variants according to Kupsch et al. (1993).

<sup>d</sup> The Opa specification for CD66 receptor is according to Gray-Owen et al. (1997b) and Bos et al. (1997).

<sup>e</sup> OpaC is bifunctional, and interacts as well with heparan sulphate (Chen et al., 1997).
suggest that these proteins mediate different cellular responses depending on the cell type and the pattern of CD66 molecules that are present on the surface. This is consistent with the fact that an enhanced oxidative burst is triggered in granulocytic cells by Opa proteins that bind CD66a.

In addition to promoting interactions with PMN, Opa-mediated binding to CD66a increases GC adherence to tumour necrosis factor (TNF)-α-activated human umbilical vein epithelial cells (HUVECs) *in vitro* (Gray-Owen *et al.*, 1997b). Opa proteins binding to CD66a allow transcytosis of GC across a monolayer of polarized T84 epithelial cells (Wang *et al.*, 1998).

Recently, another possible ligand for Opa proteins was identified by using the yeast two-hybrid system. Williams *et al.* (1998) showed that one Opa protein binds to pyruvate kinase. Subsequently, these authors have demonstrated that intracellular gonococci bind pyruvate kinase and require host pyruvate for growth. A similar function for meningococcal Opa proteins has not yet been reported.

### Opa and Opc proteins in Neisseria meningitidis

Opc and class 5 proteins mediate an interaction between meningococci and eukaryotic cells, only in a non-encapsulated background. In capsulated bacteria, Opa and Opc do not seem to affect bacterial interactions with host cells. In addition class 5- and Opc-mediated cellular interactions are also profoundly inhibited by lipooligosaccharide (LOS) sialylation (Virji *et al.*, 1992, 1993; de Vries *et al.*, 1998).

Opa proteins allow MC interaction with epithelial cells, PMN and to a lesser extent with endothelial cells. It has been established that Opa proteins bind members of the CD66 family, thus mediating adhesion to and invasion of epithelial cells (Virji *et al.*, 1996a, b). In the case of interactions with PMN, binding to CD66 leads to phagocytic elimination of Opa-producing MC.

Opc significantly increases adhesion and invasion of bacteria into both Chang cells and HUVECs (Virji *et al.*, 1992), however, only when present at high levels (Sarkari *et al.*, 1994). Opc causes adherence of non-piliated MC and promotes 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 (de Vries *et al.*, 1998). However, the exact nature of the proteoglycan receptors recognized by these adhesins/invasins is unknown. Opc has also been reported to facilitate adhesion to and invasion of endothelial cells through the binding of vitronectin in a trimolecular complex. The Opc-producing meningococci interact with vitronectin and use it to attach to the integrin αvβ3 on the apical surface of the endothelial cells (Virji *et al.*, 1994). This raises the question of functional similarities between meningococcal Opc and gonococcal OpaA (see above), especially when considering that a MC equivalent of GC OpaA has not yet been found. However, in strains lacking Opc, MC ability to bind to proteoglycan may be carried by some other components, including an Opa protein.

### Porins

Pathogenic *Neisseria* produce two porins designated PorA and PorB (Table 2). Neisserial porins have been shown to translocate spontaneously as functional voltage-gated ion channels into plasma membranes of eukaryotic cells, causing a transient change in membrane potential and interference with cell signalling (Ulmer *et al.*, 1992). Furthermore, the *porB* gene of GC but not *porB* genes is found only in GC and are mutually exclusive, therefore a strain produces either one of these porins. These proteins are formerly designated P.IA and P.IB. PorBa and PorBb are present in MC and are also mutually exclusive, the proteins encoded by these alleles were formerly classed as Opa protein.

Table 2. Classification of neisserial porins.a,b,c

<table>
<thead>
<tr>
<th>Neisseria meningitidis</th>
<th>Neisseria gonorrhoeae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>PorA</em></td>
<td><em>PorB</em></td>
</tr>
<tr>
<td>Present</td>
<td>Pseudogene</td>
</tr>
<tr>
<td>porB1a or porB1b</td>
<td>porB2 or porB3</td>
</tr>
</tbody>
</table>

a. This table is according to Feavers and Maiden (1998).
b. A *porA* gene is present only in *N. meningitidis*. In this latter species two porins PorA and PorB may be concomitantly expressed. A *porA* pseudogene has recently been described in GC, but no *PorA* protein is produced.
c. Four *porB* alleles have been described: *porB1a*, *porB1b*, *porB2*, and *porB3*. *PorB1a* and *PorB1b* are found only in GC and are mutually exclusive, therefore a strain produces either one of these porins. These proteins are formerly designated P.IA and P.IB. *PorB2* and *PorB3* are present in MC and are also mutually exclusive, the proteins encoded by these alleles were formerly classed as class 2 and class 3.

Pathogenic *Neisseria* interactions with host cells 1127

Porins

Other bacterial components

Several other bacterial components modulate bacteria–cell interactions. Sialylation of LOS can modulate Opa- and/or Opc-mediated interactions negatively (van Putten, 1993; de Vries *et al.*, 1998). Similar observations were made with the MC capsule (Nassif *et al.*, 1994). Other adhesins apart from those reported above have also been described. For example, GC produces a 36 kDa outer membrane protein with a binding specificity
for a gangliotetraosylceramide. Several loci of both pathogenic Neisseria recognizing glycolipids and allowing binding to human epithelial cells have been identified (Paruchuri et al., 1990; Schwan et al., 1998). In addition, an unidentified inducible gonococcal adhesin that binds the luteotropin receptor has been described (Spence and Clark, 1997).

Regarding intracellular survival, IgA1 protease, which is believed to be crucial for mucosal colonization, has recently been shown to be important for intracellular survival. Hence, it cleaves the LAMP1 protein and therefore prevents phagolysosomal fusion, thus playing a role in the ability of pathogenic Neisseria to survive inside epithelial cells (Lin et al., 1997; Ayala et al., 1998).

Is it possible to assemble the puzzle?

Gonorrhoea

The events that led to the development of gonorrhoea were initially worked out using ex vivo organ culture models (McGee et al., 1981; McGee et al., 1983; Harvey et al., 1997). Bacteria were shown to adhere to the microvilli of non-ciliated cells of the epithelium. They induce focal polymerization of actin, often accompanied by microvilli extensions, enter the apical pole of epithelial cells and transcytose to the basolateral side of the cells. Once in the subepithelial space, they induce the inflammatory process responsible for the disease symptoms. It is tempting to speculate that the initial contact is established by bacterial pilus and is then followed by a tight adherence via Opa proteins. This Opa-mediated interaction could promote actin polymerization followed by transcytosis through epithelial cells. Considering the data obtained using a monolayer of epithelial cells (Wang et al., 1998), binding of Opa protein to CD66 is the most likely pathway responsible for this transcytosis. In this model, pilus are colonization factors. The role of OpaA remains unclear, especially considering that heparan sulphate-containing receptors are located at the basolateral surface of polarized cells. Consequently, OpaA-mediated entry cannot be the driving force for transcytosis. On the other hand, it could be a way of amplifying the invasion of cells, once the inflammatory process has started and has led to the breakdown of the mucosal barrier.

The above model does not take into account several experimental observations. Piliated Opa+ gonococci induce cytoskeletal rearrangements (Merz and So, 1997), cross a monolayer of epithelial cells within 48 h (Merz et al., 1996) and upregulate the induction of inflammatory cytokines by epithelial cells (Naumann et al., 1997). Taken together, these observations strongly suggest that the cellular events induced by pilus-mediated adhesion are sufficient to induce the inflammatory process. This is consistent with the data obtained using a human challenge model, which show that the occurrence of the inflammatory process occurs with piliated strains (Cannon et al., 1998). These observations argue in favour of a more sophisticated role for pili than just being colonization factors. Thus, after pilus-mediated adhesion, a signal might be transduced to epithelial cells that leads to the induction of cytokine production and subsequent development of the inflammatory process. The recruitment of inflammatory cells to the subepithelial space may be responsible for the breakdown of the mucosal barrier and the passage of GC into this space. In this model, the role of Opa-mediated transcytosis and Opa-mediated cellular invasion in the events that lead to inflammation is unclear. However, in vivo, N. gonorrhoeae invades urethral epithelial cells (Apicella et al., 1996). These intracellular bacteria could correspond to the resistant forms that are protected from the bactericidal activity of the extracellular fluids. Opa-mediated invasion may therefore be a way to increase duration of the carrier state in asymptomatic patients, and subsequently to favour the transmission of the bacteria.

As far as porins are concerned, their role in nucleating actin could play a major role in the cytoskeletal modifications responsible for bacterial entry. As pointed out above, the IgA protease might be essential in intracellular survival and therefore play a major role in persistence and dissemination. This is consistent with the data obtained using the human challenge model, which show that GC mutants unable to produce IgA protease were unaffected in their ability to cause infection (Cannon et al., 1998). In the near future, the use of the human challenge model should allow the identification of the role of other bacterial factors mediating cellular interactions in gonorrhoea.

Meningococcal diseases

A major point in the life cycle of MC is its ability to colonize the nasopharynx asymptomatically. The mechanism of this colonization is unknown. MC strains colonizing the nasopharynx are mostly non- or poorly capsulated. Therefore, the factors available for cellular interactions are similar to those found in GC, and thus the mechanisms of colonization could be very similar. The question why these factors that are responsible for an inflammatory process in the genitourinary tract do not induce a similar process in the throat remain unanswered. One major difference is that the urethral epithelium is usually sterile, unlike the nasopharynx, and the signalling that occurs after bacterial colonization and that is responsible for the occurrence of the inflammatory process in the urethra may not happen in the nasopharynx. In the case of heavily capsulated pathogenic strains, the bacteria may be able to colonize the nasopharynx efficiently only with the help of bundle-forming pilus. The mechanism by which these bacteria cross the mucosal barrier is unknown; a possible
way is via the M cells present at the tonsillar sites. It is conceivable that transcytosis through these cells may be an efficient route of invasion for MC, as described for enteric pathogens (Jones et al., 1994).

Another situation in which MC interacts with a cellular barrier occurs when it crosses the blood–brain barrier. At this stage MC is capsulated and pili seem to be the only bacterial factor that can mediate any cellular interaction. The blood–brain barrier is composed of epithelial and/or endothelial cells that have tight junctions, which limits paracellular flux. Recent in vivo data have demonstrated that MC is capable of interacting 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 (Pron et al., 1997). However, a better understanding of all of the steps involved in the movement of MC across the blood–brain barrier will require the development of in vitro models. Because the main cellular characteristic of the blood–brain barrier is the existence of tight junctions that limit paracellular flux, the in vitro models used to study this step should be either a monolayer of tight junction-forming endothelial cells and/or epithelial cells. Even though considerable progress has been made in our understanding of the blood–brain barrier, models using human brain endothelial cells are not yet available. This has led to the use of epithelial monolayers of human polarized cells with organized tight junctions similar to those responsible for the blood–brain barrier. Using such a model, which most likely reflects only some aspects of the movement of MC across the blood–brain barrier, MC has been shown to cross a monolayer via the transcellular route without destroying the intercellular junctions (Pujol et al., 1997). It should be pointed out that even though bacteria are not been seen between cells, this does not exclude the possibility that this route is used. Initially, the adhesion of MC 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 MC covering the cells, thus realizing a diffuse adherence phenotype. At this stage, bacteria adhere intimately and firmly to the apical membrane and in some places are found intracellularly.

Knowledge of the bacterial factors involved in all these steps is very incomplete (Fig. 1). As mentioned, pilus-mediated adhesion is probably most critical during the first step of localized adherence. At the diffuse adherence step, MC has lost its pili. There are several possible explanations for this process. Firstly, cross-talk between bacteria and cells could lead to the downregulation of genes involved in pilus biogenesis. Secondly, pili could be retracted, thus bringing into contact the outer membrane of the bacteria and the cellular plasmic membrane. The loss of piliation after the localized adherence step suggests that pili per se are not involved in the intimate attachment observed during the diffuse adherence. The best candidates for this function would be the Opa/Opc proteins. However, several observations are not consistent with a role for these molecules in this intimate attachment: (i) opc isolates are capable of intimate attachment (Pujol et al., 1997); (ii) a naturally occurring Opa~ variant is also able to undergo intimate attachment (Pujol et al., 1997). The role of porins in this process remains to be assessed. It should be pointed out that an inoculum of non-piliated bacteria is incapable of diffuse adherence, suggesting that the initial phase of localized adherence is necessary for the production of some as yet unidentified factors that lead to a diffuse adherence phenotype. Furthermore, there is evidence that PilT, a cytoplasmic nucleotide-binding protein, involved in pilus retraction, is required to disperse bacteria from the localized to the diffuse adherence pattern, and to induce the loss of piliation and intimate attachment (Pujol et al., 1999). Pili could be seen as sensory organs that, once they recognize their receptor on the surface of the cells, transduce a signal to the bacteria.
which in turn upregulate the expression of some unidentified component(s) necessary for intimate attachment and expression for the signalling events linked to this step. The loss of piliation at the diffuse adherence step could result from the same signalling event. The cellular mechanisms driving the transcytosis of bacteria remain unexplored.

One of the most fascinating aspects of the study of the pathogenesis of *N. meningitidis* and *N. gonorrhoeae* is that they have evolved common mechanisms of interaction with cells, even though they clearly have a different pathogenesis. The question of whether additional factors are responsible for the specificity of the pathogenesis of each bacteria is unanswered and will benefit from the availability of the sequence of the meningococcal genome and its comparison with that of *N. gonorrhoeae*. On the other hand, the similarity in the bacterial attributes evolved by these pathogens suggests that bacterial cell interactions may be essential not only for pathogenesis but also for some other aspects of the life cycle of pathogenic *Neisseria*-like survival and dissemination.

**Acknowledgements**

We are grateful to C. Tinsley for careful reading of this manuscript. We wish to thank the anonymous reviewers for their helpful suggestions in improving this manuscript. The work in the laboratory of XN is supported by INSERM, Université René Descartes Paris 5, and the Fondation pour la Recherche Médicale.

**References**


© 1999 Blackwell Science Ltd, Molecular Microbiology, 32, 1124–1132


Stimson, E., Virji, M., Makepeace, K., Dell, A., Morris, H.R.,


