Interactions between *Neisseria meningitidis* and human cells that promote colonisation and disease

Anne Corbett, Rachel Exley, Sandrine Bourdoulous and Christoph M. Tang

Neisseria meningitidis **is the leading cause of bacterial meningitis, a potentially fatal condition that particularly affects children. Multiple steps are involved during the pathogenesis of infection, including the colonisation of healthy individuals and invasion of the bacterium into the cerebrospinal fluid. The bacterium is capable of adhering to, and entering into, a range of human cell types, which facilitates its ability to cause disease. This article summarises the molecular basis of host–pathogen interactions at the cellular level during meningococcal carriage and disease.**

Neisseria meningitidis is a Gram-negative diplococcal bacterium that is the leading cause of purulent meningitis, which particularly affects children (Ref. 1). The most critical outcome of infection is septicaemia, which carries a 10% mortality rate and the risk of physical disability, such as loss of limbs or digits (Ref. 2). Although isolated meningeal disease is usually less serious than septicaemia, patients may still be left with permanent neurological sequelae including

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Interactions between *Neisseria meningitidis* and human cells that promote colonisation and disease

Accession information: DOI: 10.1017/S1462399404008087; Vol. 6; Issue 14; 9 July 2004
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https://doi.org/10.1017/S1462399404008087 Published online by Cambridge University Press

deafness. The bacterium is transmitted from person to person by aerosol spread among healthy carriers; *N. meningitidis* is found in the nasopharynx of 3–40% of asymptomatic individuals (Refs 3, 4), with bacteria detectable deep to the epithelial layer (Ref. 5). The bacterium is an obligate commensal of the human upper airway, which is the only known reservoir of infection. Therefore, colonisation is both necessary for the propagation and survival of the bacterium, and is the first step in the disease process.

As with other host-adapted microbes, biologically relevant systems for studying pathogenesis have been difficult to establish. The general approach taken has been to use specific models to reflect individual stages in the progression of infection, as no single assay replicates the multiple steps in pathogenesis. Much emphasis has been placed on examining the interaction between the bacterium and isolated, immortalised epithelial cells for understanding colonisation. Rodent models have been adapted to investigate the dissemination of the bacterium in the systemic circulation (Refs 6, 7, 8). Experiments with endothelial and meningeal cells have been used to represent the subsequent stage of attachment to endovascular cells and entry into the cerebrospinal fluid (CSF), leading to meningitis. Although none of these models is ideal, they have provided valuable insights into the bacterial factors required for pathogenesis and their cellular targets.

The purpose of this article is to review what has been learnt about the interaction between the bacterium and host cells, particularly the non-professional phagocytes (epithelial and endothelial cells) that are crucial to colonisation and are the probable route of spread to the CSF. Several adhesive factors have been characterised and their cognate receptors defined. However, far less is known about the downstream effects that follow engagement of receptors with these adhesins, or how the bacterium traverses cellular barriers.

Adhesins expressed by *N. meningitidis*

In order to colonise the nasopharynx effectively, enter the systemic circulation and cause disease, *N. meningitidis* interacts with many different cell types. Common themes have emerged from studies employing different cell types, and it appears that the bacterium expresses several adhesins that mediate association with a range of host cells (Fig. 1). Some mechanisms by which *N. meningitidis* attaches to endothelial cells also apply to the behaviour of the related pathogen, *Neisseria gonorrhoeae*, with epithelial cells from the urogenital tract. However, care must be taken not to extrapolate all findings between these pathogens. There is considerable difference in the gene content between the two species; for instance, *N. gonorrhoeae* has around 400 genes that are not found in *N. meningitidis* (Jason Hinds, St George's Hospital, London, pers. commun.). Furthermore, unlike *N. meningitidis*, the gonococcus does not express a polysaccharide capsule (Ref. 9). This might affect the accessibility of bacterial ligands to host receptors, and is especially relevant for serogroups of *N. meningitidis* (B and C) that express a capsule of polymers of sialic acid that is also present on human cells (Ref. 10).

Another important consideration is the extensive *N. meningitidis* strain-to-strain variation in the surface structures, including the capsule, lipopolysaccharide (LPS) and outer membrane proteins (Refs 11, 12, 13). Indeed, *N. meningitidis* strains are grouped into 12 serogroups on the basis of their capsular polysaccharides; the most important serogroups associated with disease in humans are A, B, C, Y and W135 (see Todar's online textbook of Bacteriology; http://textbookofbacteriology.net/neisseria.html). These differences in surface structures affect the binding capacity of isolates (Ref. 14), and might influence the comparability of results from different groups. The problem is further compounded by variation arising within an individual isolate (Fig. 2). An important feature of *N. meningitidis* revealed by genome sequencing is the extent of repetitive DNA and the mechanisms for phenotypic variation (Ref. 15). There are examples of transposition that directly affect gene expression (Ref. 11) or contain potential promoters (Ref. 16). Furthermore, variation in the sequence of surface antigens can occur through gene conversion (Ref. 17). For example, although the pilin subunit is expressed from a single locus, the allele may be replaced with others in the chromosome (Fig. 2). On:off phase variation occurs through changes in the length of homopolymeric tracts through slip-strand mispairing (Fig. 2). This can lead to an alteration in the structure of a promoter (affecting transcription) or the introduction of a frameshift mutation (resulting in a truncated protein product) (Refs 11, 18). Finally, point mutation can

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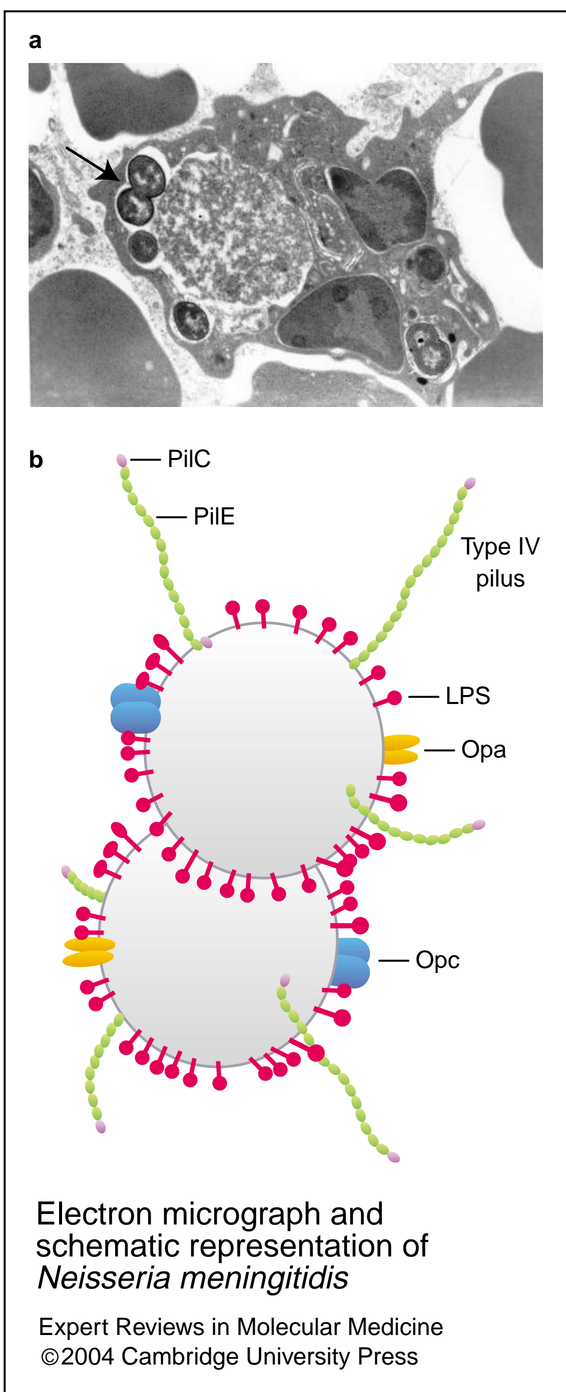

Figure 1. Electron micrograph and schematic representation of *Neisseria meningitidis*. (a) Electron micrograph showing paired bacteria (arrow) within a splenic macrophage. Approximate magnification = x10 000. (b) Schematic representation showing the major adhesins. Pathogenic *N. meningitidis* possesses several surface-expressed adhesins. The type IV pilus mediates initial interactions with host cells. The opacity proteins Opa and Opc are phase-variable outer membrane proteins believed to play a role in later stages of adhesion and invasion, and enable adhesion in the absence of pili. The lipopolysaccharide (LPS) molecules display variation in side chains; the unsialylated forms of LPS might contribute to adhesion by binding to asialoglycoprotein receptors. The polysaccharide capsule is believed to inhibit adhesion to cells, possibly due to negatively charged molecules such as sialic acid that repel host cells or by masking the exposure of other surface adhesins.

also generate phenotypic diversity in populations of *N. meningitidis*, especially in strains that are defective for DNA adenine methylation mismatch repair (Ref. 19). Therefore, analysing variants selected for desired characteristics and mutants derived from single colonies can prove difficult to interpret, and may be a further reason for discrepancies between laboratories. Backcrossing and complementation are necessary to be certain that a phenotype results from a particular mutation.

Type IV pili and receptors

The major adhesin involved in the initial attachment to many host cells is the type IV pilus (Tfp) (Fig. 1b). Pili are classified according to functional and structural characteristics. In Tfp, the pilus subunits have a distinctive structure, including a short positively charged leader peptide that is cleaved during assembly, N-methylphenylalanine as the first residue in the mature protein and a highly conserved hydrophobic N-terminal region. Structural and antigenic variation within and between species occurs in the C-terminal two-thirds of the protein. Tfps are expressed by several pathogenic bacteria including enteropathogenic *Escherichia coli* (Ref. 20), *Vibrio cholerae* (Ref. 21) and *Pseudomonas aeruginosa* (Ref. 22). The Tfp of pathogenic *Neisseria* is a dynamic filament 6 nm in diameter; it is essential for adhesion and is involved in bacterial motility and competence for DNA uptake, and thence genetic transformation (Ref. 23). Tfps are formed from multiprotein complexes, with the pilus filament composed of a multimer of a 18–22 kDa pilin subunit protein encoded by *pilE* (Ref. 24). The N-terminus of the protein forms a hydrophobic tail that coils into the central core of the filament to provide tensile strength (Ref. 25). The C-terminus forms a globular head that contains a variable loop exposed on the external surface of the fibre.

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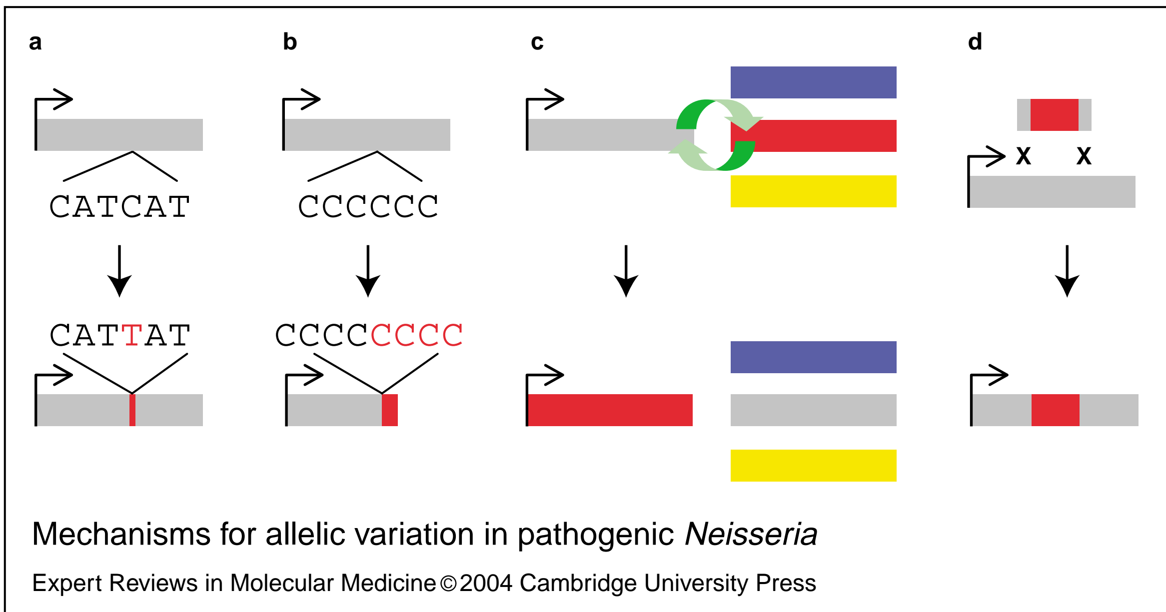

Figure 2. Mechanisms for allelic variation in pathogenic *Neisseria*. The sequence of a gene can be altered in several ways. (a) Point mutation can result in a different protein coding sequence or the introduction of a stop codon, resulting in a truncated protein. (b) Changes in the length of a homopolymeric tract in an open reading frame can lead to premature termination of translation and to reversible off switching. Modification of homopolymeric tracts in promoter sequences can affect the level of gene transcription (see Ref. 58 for example). (c) Intra-genomic gene conversion can result in expression of previously transcriptionally silent genes. For instance, gene conversion of transcriptionally silent *pilS* alleles into the expression locus of *pilE* results in changes in expression of the pilus subunit. (d) The competence of *N. meningitidis*, or the ability to take up DNA and become genetically transformed, leads to the acquisition of exogenous DNA and the creation of mosaic genes in vivo.

Intragenomic alteration in the sequence of the subunit occurs through *recA*-dependent gene conversion between the *pilE* expression locus and multiple, non-expressed (*pilS*, silent) copies in the genome. In the meningococcus, *pilS* genes are clustered near *pilE*; by contrast, in *N. gonorrhoeae*, the *pilS* genes are widely distributed across the genome (Ref. 26). The biological significance of this is unclear. The pilin protein can also be modified by glycosylation (Ref. 27). This substitution is subject to phase variation and does not affect the function of the Tfp in a major way, although non-glycosylated mutants have slightly enhanced binding to epithelial cells compared with glycosylated bacteria (Ref. 28).

The 110 kDa PilC protein has an important role in Tfp function (Ref. 29). There are two alleles, PilC1 and PilC2, which were originally identified in the gonococcus. The precise location of PilC on the Tfp remains unresolved. The initial description (Ref. 30) that the protein is the tip adhesin contrasts with its subsequent localisation on the outer membrane of the bacterium (Ref. 31). Although mutation in the *pilC2* gene does not affect adhesion, *pilC1* mutants do not associate with host cells (Ref. 32). Expression of both *pilC* alleles is subject to phase variation as a consequence of homopolymeric tracts within the open reading frames (see Fig. 2). Thus, isolates can lose expression of this important protein following passage in vitro. Sequence analysis of the *pilC* genes has revealed differences in the promoter region, indicating that they might be under independent transcriptional control (Ref. 33). Reporter *lacZ* fusions with the *pilC* genes showed that *pilC1* is upregulated following infection of host cells, a response that is not seen for *pilC2*. Upregulation was necessary for full adhesion to human cells (Ref. 34), including endothelial cells, and was detected in the CSF and choroid plexus, supporting a role for this protein during the development of meningitis (Ref. 35).

Another pilus component, PilT, was shown initially to be required for twitching motility and competence in the gonococcus (Ref. 36). The protein is also required for association with

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epithelial cells after initial attachment at a stage called intimate attachment (Ref. 37). PilT is now known to be necessary for the retraction of pili, which is thought to be the motive force that opposes the pathogen in close association with the host cell (Ref. 38).

The proposed host cell receptor for Tfp is CD46 [membrane cofactor protein (MCP); reviewed by Ref. 39)]. This receptor is a member of a superfamily of complement resistance proteins, of which at least six isoforms have been described; members of the family are expressed in nearly all human cell types, except erythrocytes. CD46 is a well-characterised receptor protein for several viruses including measles (Ref. 40) and human herpes virus 6 (Ref. 41). For *N. meningitidis*, purified pili appear to bind to a CD46-sized protein in western blotting, and anti-CD46 antibodies block *N. meningitidis* adhesion to host cells (Ref. 42). Further evidence has been provided by showing that *Staphylococcus aureus* coated with a CD46 fusion protein are aggregated rapidly by piliated bacteria, whereas non-piliated cultures have no effect (Ref. 42). However, there is some doubt about whether CD46 is the cognate receptor for Tfp, as meningococcal adhesion to CD46 ectopically expressed on Chinese hamster ovary cells is weak compared with adhesion to human epithelial or endothelial cells (Ref. 42), and there is an inverse correlation between the level of CD46 expression by cell lines and the degree of adhesion with piliated *N. gonorrhoeae* (Ref. 43). In addition, *N. meningitidis* does not interact with some human cells, such as human primary astrocytes, even though these cells express the different CD46 isoforms (S. Bourdoulous et al., unpublished). To study the CD46–Tfp interaction further, transgenic mice expressing human CD46 were infected with *N. meningitidis* (Ref. 44). Interestingly, animals expressing CD46 were more susceptible to systemic challenge than control animals, although this was independent of the piliation status of the infecting bacterium. Following infection by the intranasal route, which is the site of the proposed interaction between *N. meningitidis* and CD46, only a low proportion of transgenic animals developed disease if they received piliated meningococci, whereas all mice remained well if given non-piliated organisms.

Opacity proteins: Opa and Opc

Although encapsulated meningococci adhere mainly by means of the Tfp, some strains isolated from the nasopharynx are unencapsulated and non-piliated as a result of phase variation, and must therefore rely on alternative adhesins. Under these circumstances, the opacity proteins, Opa and Opc, can mediate adhesion (Ref. 45).

The multigene Opa family has been studied extensively in the gonococcus, and is also present in the meningococcus (Ref. 46). Opa proteins consist of eight transmembrane β-strands and four surface-exposed loops. The transmembrane and periplasmic sections are highly conserved, whereas the first three surface-exposed loops display antigenic variation. There are 4–5 loci encoding Opa proteins, and expression varies between strains. Thus, a single bacterium might express several Opa proteins at any one time, and variation is enhanced by horizontal transfer of *opa* alleles to form mosaic genes. This has important functional consequences, as expression of different Opa proteins confers tropism to different cell lines (Refs 45, 47).

Several different Opa receptors have been identified, dependent upon which Opa is expressed by the bacterium. In the gonococcus, members of one class of Opa bind heparan sulphate proteoglycans (HSPGs) present on certain epithelial cells (Ref. 47). Two pathways then mediate entry into the cell, either through localised recruitment of lipid hydrolysis (Ref. 48) or a vitronectin-dependent mechanism (Refs 49, 50). Vitronectin is an extracellular matrix (ECM) glycoprotein that forms a bridge between Opas on the bacterial surface and integrins on host cells; this can be sufficient to facilitate bacterial uptake in certain cell lines (Ref. 51). However, the HSPG interaction may be both non-specific and mediated by porins since strains unable to express porin cannot bind HSPG (Ref. 52).

More recently, additional receptors for the Opa proteins have been identified: these are members of the CD66 carcinoembryonic antigen family that mediate cell–cell adhesion [e.g. carcinoembryonic antigen-related cell adhesion molecule (CEACAM); Ref. 53]. The receptors are transmembrane or peripheral glycoproteins and are expressed on virtually all human cells except erythrocytes (reviewed by Ref. 54). Opa proteins can bind to several members of the CD66 family (Refs 55, 56) and the Opa structure can be altered to determine CD66 specificity (Ref. 56). The host cell response varies depending on the receptor type and Opa group, but includes binding, uptake and activation of signalling pathways.

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Opc proteins share physiochemical properties and a weak homology with the Opa group, but differ in their structure and genetic control (Refs 57, 58). Unlike the Opa proteins, they are unique to *N. meningitidis*, although are only expressed by a subset of strains. The expression of Opc proteins is governed by alterations in the length of a homopolymeric tract in the promoter region (Ref. 58). The action of Opc follows interaction with the serum glycoprotein vitronectin, which acts as a molecular bridge to $\alpha_v\beta_3$ integrins on the surface of umbilical vein endothelial cells. This facilitates entry of the bacterium into these cells (Ref. 59). Opc also affects internalisation of bacteria into human brain microvascular endothelial cells; however, this is mediated through the interaction of Opc with fibronectin in serum and then $\alpha_v\beta_1$ integrins (Ref. 60).

Other adhesins

Other molecules expressed at the bacterial cell surface have also been implicated in adhesion. The structure of LPS of the meningococcus is similar to that of other members of the Enterobacteriaceae family, but lacks a repeating O-antigen. Differences in side-chain composition underlie the immunotypes of different strains (Ref. 61). Some immunotypes express the lacto-*N*-neotetraose epitope that mimics human asialocarbohydrates; indeed, the contribution of LPS to *N. gonorrhoeae* binding to the host cell may be through interaction with asialocarbohydrate receptors (Ref. 62). However, little is known about the role of LPS in the adhesion of *N. meningitidis*.

The effects of the capsule on meningococcal adhesion have been mostly studied in strains expressing the polysialic acid, serogroup B polysaccharide. The capsule is antiphagocytic and antibactericidal owing to surface-exposed polysaccharides. Thus, the capsule is universally expressed by strains isolated from the blood and CSF. The capsule reduces adhesion possibly because of negatively charged molecules such as sialic acid that repel host cells (Ref. 1). However, the enhanced interactions of capsule-negative strains in experiments with epithelial cells are difficult to interpret given the propensity of these strains to aggregate.

Molecular mechanisms underlying host cell invasion by *N. meningitidis*

Invasion of endothelial and epithelial cells

The invasion of host epithelial and endothelial cells by *N. meningitidis* appears to be essential for meningococcal pathogenesis since in vitro evidence indicates that *N. meningitidis* crosses through individual cells (transcytosis) while negotiating cellular barriers (Refs 63, 64). Therefore, adhesion and invasion of the epithelium and endothelium has been extensively studied (Ref. 65), and many of the initial processes involved are now well understood (Fig. 3).

Initially, bacteria adhere to host cells, a process mediated by the Tfp. Within 10 minutes of contact between the pathogen and cell, a transient release of calcium from intracellular stores occurs, which is thought to be mediated through pili binding to CD46 (Ref. 66). This calcium flux triggers lysosome exocytosis, which might aid the bacterium in its adhesion (Ref. 67). The calcium-influx response is dependent on the pilin subunit PilC1 and can be blocked by antibodies against the CD46 receptor (Ref. 66), indicating the vital role for PilC1 and the upregulation pathway described previously. CD46 mutants with a truncated cytoplasmic domain inhibit gonococcal adhesion, suggesting that the activation of a signalling pathway coupled to CD46 is required to initiate bacterial adhesion (Ref. 68). Moreover,

Figure 3. Schematic representation of the signalling pathways activated by *Neisseria meningitidis* during cell entry. *(Legend; see next page for figure.)* Type IV pili initiate the interaction of virulent, encapsulated *N. meningitidis* with human endothelial cells by interacting with a cellular receptor, possibly CD46 (Ref. 42) and/or a different, non-CD46, pilus receptor. (a) Through a Rho-GTPase-independent pathway, pilus-dependent adhesion induces the recruitment of ezrin and moesin, two proteins that link the cellular membrane to the actin cytoskeleton, and the clustering of several transmembrane proteins: CD46, ErbB2 and the ezrin-binding protein intercellular cell adhesion molecule 1 (ICAM-1; CD54). (b) Through a Rho-GTPase-dependent pathway, the activation of both Rho and Cdc42 GTPases induces a localised polymerisation of cortical actin. This leads to the formation of membrane projections reminiscent of epithelial microvilli structures, which surround single bacteria and provoke their internalisation within intracellular vacuoles. (c) Downstream of bacteria-induced ErbB2 activation, Src tyrosine kinase phosphorylates cortactin, an event that is involved in bacterial entry (phosphorylation denoted by P).

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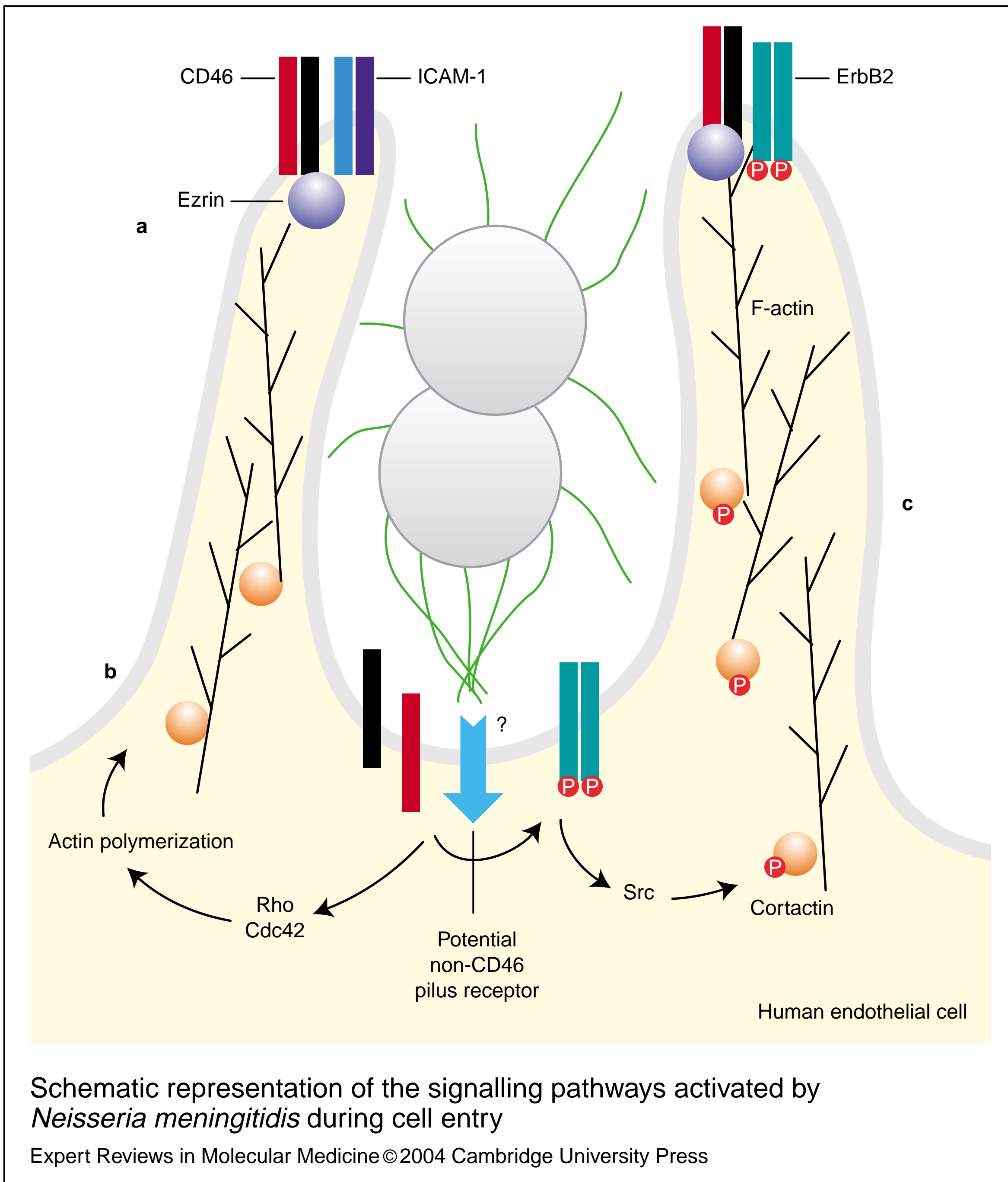

Figure 3. Schematic representation of the signalling pathways activated by *Neisseria meningitidis* during cell entry. *(See previous page for legend.)*

infection of epithelial cells by *N. gonorrhoeae* leads to the rapid tyrosine phosphorylation of CD46 by c-Yes, which is a member of the Src-family of tyrosine kinases, and inhibition of Src kinases reduces the ability of epithelial cells to support gonococci adhesion (Ref. 69). Although it has not been shown that CD46 tyrosine phosphorylation results from a direct interaction between Tfp and CD46, these data suggest that the association between *N. gonorrhoeae* and CD46 elicits a host cell response that facilitates the firm adhesion of the bacteria on host cells.

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Studies using epithelial or endothelial cells infected with *N. meningitidis* have shown that, following this initial attachment, adhesion occurs in two stages: localised adhesion (during which bacterial engulfment occurs) and diffuse adhesion (during which the bacteria disperse over the surface of the host cell) (Refs 37, 64, 70). In localised adhesion, observed four hours after infection, the bacteria proliferate at the cell surface, forming microcolonies (Ref. 64). This is accompanied by transient formation of microvilli-like structures that extend from the cell surface towards the bacteria and finally engulf the bacteria, leading to their internalisation within vacuoles (Fig. 3).

The cell membrane rearrangements require pili and PilT (Ref. 37). PilT is involved in pilus-mediated motility and pilus retraction, which is greatly enhanced when mechanical force is exerted on the host cell membrane; this process has been extensively studied in *N. gonorrhoeae*. The force exerted by pilus retraction has been measured as 80 pN or more (Ref. 38), and has been found to be in the same range (20–80 pN) as that required to induce elongation of microvilli. The microvilli-like protrusions are enriched in cortical actin, as well as in ezrin and moesin, both of which are members of a protein family involved in structuring and regulating the morphological changes of the plasma membrane (reviewed by Ref. 71). Protrusions are also enriched in the integral membrane proteins CD44 and intercellular cell adhesion molecule 1 (ICAM-1; CD54), in structures referred as cortical plaques (Refs 70, 72, 73). Interestingly, the formation of membrane protrusions was also observed in vivo, by transmission electron microscopy analysis of brain sections from a child who died from fulminant meningitis (Ref. 65). These observations strongly suggest that such morphological modifications of the host cell membrane are essential for *N. meningitidis* to cause meningitis by crossing the human vascular endothelium via a transcytosis pathway.

Activation of Rho and Cdc42 is required for cortical actin polymerisation (Ref. 70) beneath adhering meningococci during the formation of cortical plaques. These proteins are members of the Rho-family GTPases that have been implicated in the regulation of many cellular activities, particularly actin cytoskeleton organisation (Ref. 74). Recruitment of ezrin, moesin and transmembrane proteins to the site of bacterial adhesion is independent of Rho / Cdc42 activation, whereas subsequent actin polymerisation requires their activation, and the resulting cytoskeletal reorganisation is essential for bacterial internalisation (Ref. 70). Little else is known about the precise role of these proteins in mediating uptake of *N. meningitidis*.

There is also evidence for activation of an additional signalling pathway leading to engulfment of *N. meningitidis* involving the transmembrane receptor tyrosine kinase ErbB2 (Ref. 75). Bacterial infection induces clustering and phosphorylation of this receptor below microcolonies and this results in recruitment and activation of the cytosolic protein tyrosine kinase Src. In turn, Src phosphorylates cortactin, an F-actin-binding protein that regulates cortical actin assembly and organisation (Ref. 76) and is present in cortical plaques. Although inhibition of ErbB2 or Src is known to reduce bacterial internalisation dramatically, the downstream signalling events involved have not yet been elucidated.

After 8–16 hours, diffuse adherence occurs, during which bacteria disperse at the cell surface and form a monolayer, with a concomitant loss of the membrane protrusions observed during localised adhesion. Two main processes are responsible for diffuse adherence. The first is bacterial dispersal, which is mediated by PilT, and a reduction in pilus expression. The second is intimate attachment, characterised by PilC1 upregulation and capsule downregulation. This coordinated series of events is under genetic control in the bacterium, and responds to host cell binding by regulation of gene expression. Deghmane (Ref. 77) identified the region of the *pilC1* promoter involved in PilC1 upregulation in initial adhesion, naming it the CREN region (for ‘contact regulatory element of *Neisseria*’). Homology searches in *N. meningitidis* revealed that the *crgA* gene, encoding a transcriptional regulator, also possesses a CREN region and is induced upon host cell contact. Strains lacking components of CrgA exhibit reduced adhesion owing to a lack of *pilC1* induction. Further study showed that CrgA interacts with RNA polymerase (Ref. 78). This suggests a transcriptional control pathway that upregulates *pilC1* and downregulates *pilE* (the main structural unit of the pilus) and SiaD (the principle capsule biosynthesis protein), since the pili and capsule hinder attachment. This pathway is regulated by CrgA, which also controls its own transcription

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by negative feedback (Ref. 77). Twelve other genes that are also upregulated following host cell contact have been identified (Ref. 79); these possess a CREN region (also referred to as REP2) in their promoter sequences. Although the functions of most of these genes are unknown, this information confirms that the genetic processes involved in initial adhesion to epithelial and endothelial cells involve many complicated biochemical and regulatory pathways.

Adhesion is usually followed by internalisation of the bacterium, which occurs predominantly during the stage of localised adhesion (Ref. 70). Although details of the internalisation process are undetermined, it has been shown using monolayers (Refs 63, 64) or bilayers (Ref. 80) that the bacteria are able to progress through the epithelial or endothelial cells 18–40 hours post-infection (Ref. 50).

Interactions with ECM proteins

Between endothelial and epithelial layers, the invading meningococcus encounters the ECM. Traversal of this macromolecular complex is imperative for progression of infection. Although the interaction of Gram-positive bacteria with the ECM has been well studied (Ref. 81), there are few good examples in the Gram-negative field, so knowledge is extremely limited. Observational studies have described a degradation of the ECM as the pathogen interacts with it. Adhesion assays were carried out using different strains tested with ECM preparations and isolated matrix protein components (Ref. 82). Results showed that all the strains adhered to the ECM, especially to fibronectin and collagen types I, III and V. Further work involved blocking specific parts of the interacting proteins, leading to the identification of the cell-binding domain on the bacterial cell surface, and the revelation that the binding seen in the meningococcus is unlike other bacteria in that it does not involve the C- or N-terminals of the host fibronectin molecules (Ref. 82). It was also discovered that binding occurred regardless of the expression of Opa and Opc proteins. Further work is required to elucidate the mechanisms by which the bacterium invades and traverses this important layer around the vascular system, and also around the brain and spinal cord.

Invasion of the CSF

To cause bacterial meningitis, the bacterium must enter the CSF. In instances where there has been trauma, the bacterium can reach the CSF directly from the nasopharynx. However, in most individuals, infection of the CSF arises following spread in the systemic circulation. Therefore, the bacterium must first traverse the endothelial cell barrier to cause meningitis. Endothelial cells in the brain microvasculature are unlike other endothelial cells in that they form tight junctions. The bacterium must therefore traverse this cellular barrier, and this is thought to occur through the endothelial cells rather than around them. Indeed, bacteria have been detected in endothelial cells in a patient who succumbed to overwhelming bacteraemia (Ref. 65). As described above, the interaction of bacteria with endothelial cells is under active investigation, and many of the characteristics of interactions between *N. meningitidis* and epithelial cells also hold true for cells of endothelial origin. However, some of the proteins available within serum differ from those at the mucosal surface. For instance, Opc, which functions as a bacterial adhesin to brain microvascular endothelial cells, binds to integrins through a fibronectin, rather than a vitronectin, bridge (Ref. 60). An alternative route to the CSF involves passage via the choroid plexus. This region is responsible for the production of CSF and is located within the lateral ventricles of the brain. The endothelial layer at this site does not form tight junctions, though a cellular barrier is provided by underlying epithelial cells (Ref. 83). The role of these epithelial cells in bacterial infection has not been studied in detail.

Conclusions: implications for future treatment of meningitis?

Systemic infection by the pathogen *N. meningitidis* is a highly complex process, involving a wide array of bacterial components and activities, and interactions with host cells and host immune responses. As a result of the close co-evolution between the bacterium and humans, the pathogen has adapted to adhere to and invade many different host cells, and to avoid others. The bacterium is dynamic and adaptable, and modifies components of its outer membrane, and thence its affinity for host cells. Although we have a detailed understanding of the initial process of bacterial attachment and entry to epithelial and endothelial cells, our knowledge of subsequent steps is limited. This reflects the difficulty in establishing relevant models that mimic events beyond interactions with host cells. Nevertheless,

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nasopharyngeal tissue can be maintained in the laboratory and can be successfully infected with the meningococcus. This organ culture system has the advantage of presenting the bacterium with the organisation and range of cell types and ECM proteins encountered in the host; for instance, it has been demonstrated that there is a relationship between disease-causing clones of *N. meningitidis* and their propensity to invade the organ culture (Ref. 84). Although the model is not amenable for dissecting precise molecular interactions, it might prove a valuable resource for identifying novel bacterial factors involved in successful colonisation (Ref. 84).

A more complete understanding of *N. meningitidis* pathogenesis, particularly during colonisation, might lead to a new generation of vaccines that block the ability of the bacterium to cause disease. A similar approach of targeting adhesins has been successfully employed in the acellular pertussis vaccine (which includes the adhesin, pertactin) against the highly contagious whooping cough caused by *Bordetella pertussis*, and is being explored for preventing urinary tract infections caused by *E. coli* (Ref. 85). It is hoped that colonisation-blocking vaccines against the meningococcus will lead to the eventual eradication of this feared disease.

Acknowledgements and funding

C.M.T. is supported by the Wellcome Trust and the Meningitis Research Foundation. We thank our anonymous peer reviewers for their comments.

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https://doi.org/10.1017/S1462399404008087 Published online by Cambridge University Press

Further reading, resources and contacts

Neisseria.org provides an electronic resource for all individuals in the research community interested in research and clinical aspects of the *Neisseria*. Links are provided to information on genome sequencing, vaccine projects and other sites:
http://neisseria.org/

The Meningitis Research Foundation in the UK provides information on symptoms, vaccines and many other aspects of meningococcal disease:
http://www.meningitis.org/

The Comprehensive Microbial Resource (CMR) of The Institute for Genomic Research (TIGR) is a tool that allows the researcher to access all of the bacterial genome sequences completed to date. The genome page for *N. meningitidis* serogroup B, sequenced at TIGR, displays the circular genome with gene families highlighted. Close-up sections of the genome can be enlarged and links are provided to the full sequence of specific genes held on the SWISS-PROT database:
http://www.tigr.org/tigr-scripts/CMR2/GenomePage3.spl?database=gnm

The Sanger Institute has sequenced *N. meningitidis* serogroups A and C, and provides gene maps, lists of genes and searching of the sequence:
http://www.sanger.ac.uk/Projects/N_meningitidis/

The Gonococcal Genome Sequencing Project at the University of Oklahoma has sequenced *N. gonorrhoeae* and provides a circular gene map of *N. gonorrhoeae*, with searching of the full sequence by keyword:
http://www.genome.ou.edu/gono.html

Features associated with this article

Figures

Figure 1. Electron micrograph and schematic representation of *Neisseria meningitidis*
Figure 2. Mechanisms for allelic variation in pathogenic *Neisseria*
Figure 3. Schematic representation of the signalling pathways activated by *Neisseria meningitidis* during cell entry

Citation details for this article

Anne Corbett, Rachel Exley, Sandrine Bourdoulous and Christoph M. Tang (2004) Interactions between *Neisseria meningitidis* and human cells that promote colonisation and disease. Expert Rev. Mol. Med. Vol. 6, Issue 14, 9 July, DOI: 10.1017/S1462399404008087

Accession information: DOI: 10.1017/S1462399404008087; Vol. 6; Issue 14; 9 July 2004
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