Pathogenic neisseriae: surface modulation, pathogenesis and infection control

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Abstract | Although renowned as a lethal pathogen, *Neisseria meningitidis* has adapted to be a commensal of the human nasopharynx. It shares extensive genetic and antigenic similarities with the urogenital pathogen *Neisseria gonorrhoeae* but displays a distinct lifestyle and niche preference. Together, they pose a considerable challenge for vaccine development as they modulate their surface structures with remarkable speed. Nonetheless, their host-cell attachment and invasion capacity is maintained, a property that could be exploited to combat tissue infiltration. With the primary focus on *N. meningitidis*, this Review examines the known mechanisms used by these pathogens for niche establishment and the challenges such mechanisms pose for infection control.

Neisseria meningitidis (meningococcus) and *Neisseria gonorrhoeae* (gonococcus), the well known agents of epidemic meningitis and gonorrhoea, respectively, are related Gram-negative bacteria that specifically infect humans; both pathogens prefer to inhabit distinct human mucosal niches and cause markedly different diseases (FIG. 1). One important difference between the pathogens is that almost all clinically important *N. meningitidis* strains are encapsulated, whereas *N. gonorrhoeae* strains lack capsule biosynthetic genes. *N. meningitidis* is a frequent asymptomatic colonizer of the human upper respiratory tract, and most adults are resistant to infection through acquired immunity. However, in susceptible individuals *N. meningitidis* can cause serious blood and brain infections that are usually manifested as meningitis and septicaemia. It also seems that meningococcal strains vary in their ability to cause sporadic or epidemic outbreaks. The outcomes of meningococcal infection may be devastating and, in the absence of timely intervention, can lead to neurological disorders and death[1]. *N. gonorrhoeae* is a sexually transmitted pathogen that primarily infects the urogenital tract, giving rise to intense local inflammation and a range of clinical manifestations[2]. A signature property of the two pathogens is their ability to modulate their surface antigenic make up with remarkable speed. This is the basis of their success as human-specific pathogens, as constant surface modulation[3–5] and point mutations[6] enable the bacteria to evade human immune mechanisms. Extensive surface variation also poses a substantial problem in developing effective vaccines against several strains of *N. meningitidis* and against *N. gonorrhoeae*. Although multicomponent vaccines are being developed, the available vaccines fall short of combating all virulent strains[7,8].

An array of molecules is produced by bacteria to enable them to colonize and/or infect the host, including adhesins, which are key factors that are required for initial colonization of human mucosal sites. Characteristically, pathogens can modulate the expression and structure of adhesins and still maintain the ability to bind to mucosal epithelial cells for colonization. This might suggest some degree of structural conservation, a property that could be exploited for the prevention of infection. To achieve this aim, a thorough understanding of the range of host targeting strategies of the pathogens and of host factors that increase susceptibility to infection is needed. This Review describes the scale of the problem, focusing on our current understanding of key aspects of the pathogenic tactics of *Neisseria* spp., particularly cellular adhesion and invasion mechanisms. I also discuss the relationship between colonization and immune evasion strategies and address host susceptibility in the context of adhesion receptors. As carriage is itself considered an immunizing event that helps maintain long-term memory, the approaches that could control infection without eliminating colonization are also discussed.

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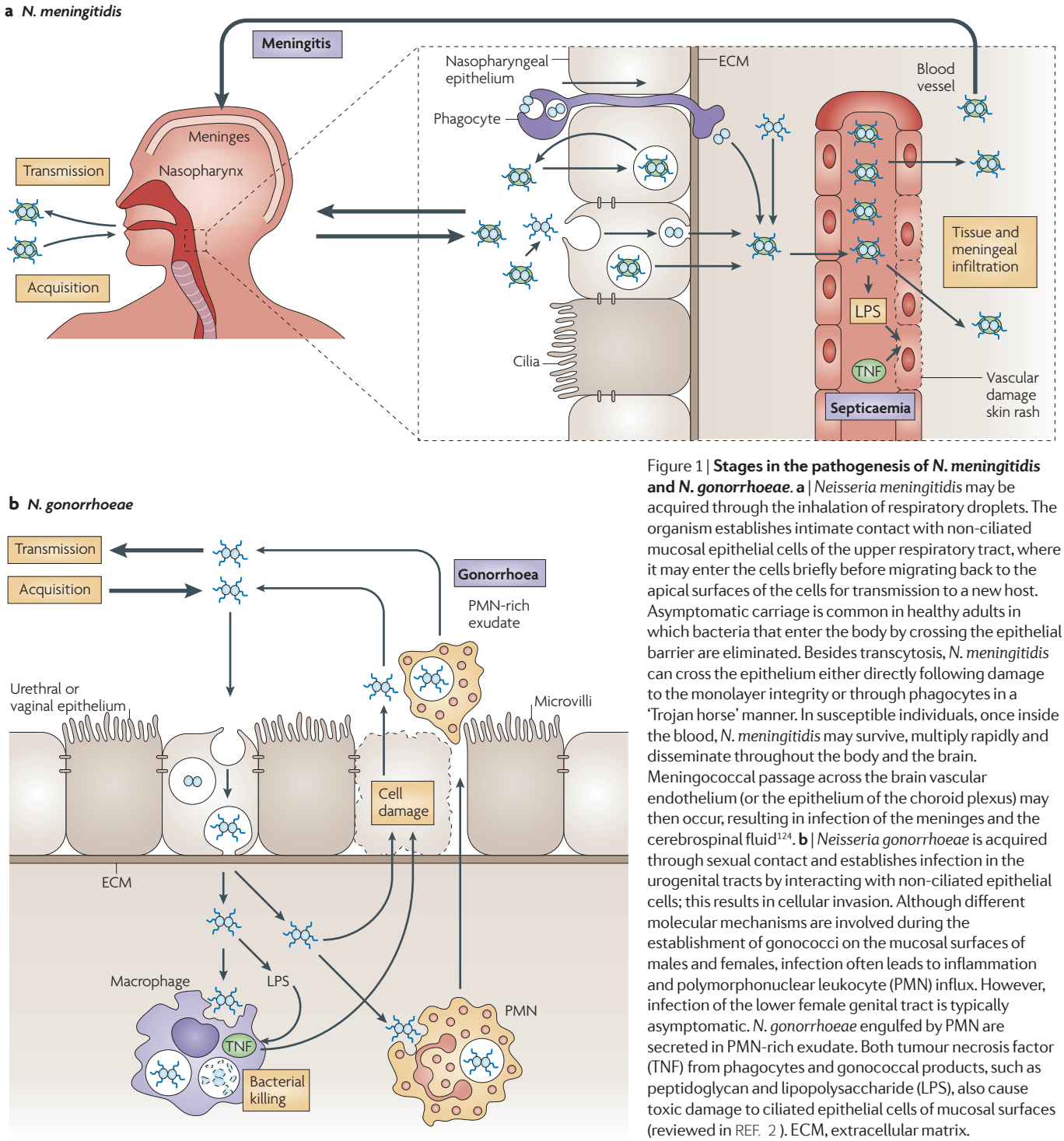


Figure 1 | **Stages in the pathogenesis of *N. meningitidis* and *N. gonorrhoeae*. a** | *Neisseria meningitidis* may be acquired through the inhalation of respiratory droplets. The organism establishes intimate contact with non-ciliated mucosal epithelial cells of the upper respiratory tract, where it may enter the cells briefly before migrating back to the apical surfaces of the cells for transmission to a new host. Asymptomatic carriage is common in healthy adults in which bacteria that enter the body by crossing the epithelial barrier are eliminated. Besides transcytosis, *N. meningitidis* can cross the epithelium either directly following damage to the monolayer integrity or through phagocytes in a 'Trojan horse' manner. In susceptible individuals, once inside the blood, *N. meningitidis* may survive, multiply rapidly and disseminate throughout the body and the brain. Meningococcal passage across the brain vascular endothelium (or the epithelium of the choroid plexus) may then occur, resulting in infection of the meninges and the cerebrospinal fluid[124]. **b** | *Neisseria gonorrhoeae* is acquired through sexual contact and establishes infection in the urogenital tracts by interacting with non-ciliated epithelial cells; this results in cellular invasion. Although different molecular mechanisms are involved during the establishment of gonococci on the mucosal surfaces of males and females, infection often leads to inflammation and polymorphonuclear leukocyte (PMN) influx. However, infection of the lower female genital tract is typically asymptomatic. *N. gonorrhoeae* engulfed by PMN are secreted in PMN-rich exudate. Both tumour necrosis factor (TNF) from phagocytes and gonococcal products, such as peptidoglycan and lipopolysaccharide (LPS), also cause toxic damage to ciliated epithelial cells of mucosal surfaces (reviewed in REF. 2). ECM, extracellular matrix.

Antigenic relatedness and carriage

Together with 17 other species, *N. meningitidis* and *N. gonorrhoeae* belong to the genus *Neisseria*[9]. Most species within the genus are classified as true human commensal bacteria and have negligible infection rates. The best recognized species of this group is *Neisseria lactamica*, which shares the human respiratory niche and antigenic structures with *N. meningitidis*. The highest carriage rate of *N. lactamica* occurs in early childhood and has been associated with the development of a cross-protective immunity against *N. meningitidis*[10]. Meningococcal carriage rate increases gradually after birth and reaches a peak in teenagers, with the average carriage rate being about 10% of the population in the United Kingdom[1]. Carriage rates tend to be high in institutional settings, for example, in military recruits and university students[11]. Thus, nasopharyngeal colonization with *N. lactamica* or other non-pathogenic *Neisseria*

Commensal bacterium
A bacterium that inhabits a host without apparent adverse effects to the host.

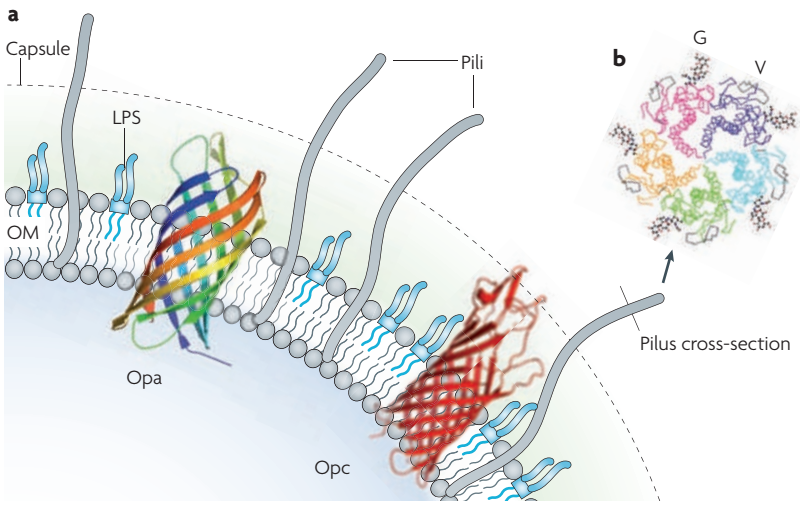


Figure 2 | **Prominent outer-membrane components of *N. meningitidis* that influence bacterial interactions with host cells. a** | Pili traverse the capsule and are the most prominent adhesins of encapsulated *Neisseria meningitidis*. In addition, the integral outer membrane (OM) adhesins, Opa and Opc, are known to mediate interactions with specific host-cell receptors in appropriate phenotypes[83]. Lipopolysaccharide may interfere with the adhesion functions of OM proteins, but can also contribute to cellular interactions by interacting with various cellular receptors[2,38]. The OM of *N. gonorrhoeae* differs in two important aspects: gonococci are non-encapsulated and Opc expression has not been shown at the protein level. **b** | A cross-section of a pilus fibre showing that variable domains (V) and glycans (G) as well as other substitutions (not shown) are located externally, whereas the constant domains are buried within the fibre, protected from the host environment. The structural model shown for Opa is that of neisserial surface protein A (NspA)[125], which like Opa is an eight-stranded β-barrel molecule, and was provided by R. L. Brady, University of Bristol, UK. The Opc model was provided by J. P. Derrick, University of Manchester, UK, and the pilus cross-section structure was provided by A. Hadfield, University of Bristol, UK.

strains does not seem to protect against *N. meningitidis* carriage, but does protect against *N. meningitidis* infection. Indeed, infection rate with *N. meningitidis* is considerably lower (1–5 per 100,000 individuals in Europe) than carriage rate. In addition, infection rate is often associated with increased susceptibility in immunocompromised hosts[1] (for example, following splenectomy or exposure to enteric pathogens that give rise to a cross-reacting but blocking IgA response) or in genetically predisposed hosts (particularly those with antibody and complement deficiencies)[11,12]. Other factors that may contribute to host susceptibility are considered below.

Meningococcal disease is a worldwide problem and is endemic in most countries. In endemic situations, it is prevalent in two age groups: children under 1 year of age and young adults between 15–19 years of age. In addition, periodic epidemics occur in Sub-Saharan Africa, especially in the 'meningitis belt' (REF. 13).

Compared with *N. meningitidis*, which spreads by respiratory aerosol droplets and can infect those in close proximity, the gonococcal mode of transmission limits the population at risk. However, after sexual contact with an infected partner, the risk of female infection is much greater than the risk of male infection, aided partly by the ability of *N. gonorrhoeae* to bind to human sperm[2]. Urogenital surfaces are the primary sites of infection by *N. gonorrhoeae*, although other sites may also become involved. Gonococcal infections are usually localized and elicit an intense inflammatory response that gives rise to purulent discharge in male patients, a hallmark of gonorrhoea. In females, the different embryological origin of the urogenital tract results in a different mode of infection, which is often asymptomatic (reviewed in REF. 2).

Virulence genes of neisseriae

Complete nucleotide sequences of several pathogenic *Neisseria* strains and of *N. lactamica* (some of which have been available for almost a decade) have facilitated the identification of numerous previously unknown putative adhesins and virulence factors[14–16] (also see the Sanger Institute *Neisseria lactamica* website and the University of Oklahoma *Neisseria gonorrhoeae* Genome Sequencing Strain FA website). A number of islands of horizontally transferred DNA have been found in the genome of *N. meningitidis*. However, no classic organized pathogenicity islands are present that define the virulent behaviour of the organisms[17]. Instead, the *N. meningitidis* genome has 'genetic islands' with identifiable genes that differ in their GC content and codon usage, which have been acquired through horizontal exchange with other mucosal bacteria[14,17]. Free exchange between genes (gene conversion) both within and between the genomes of *Neisseria* spp.[3] is a prominent mechanism for the acquisition of new traits and is facilitated by the natural competence of *Neisseria* species. Neisserial DNA in the environment is believed to arise by autolysis and by a recently identified *N. gonorrhoeae* type IV secretion system that actively transports DNA out of the cell[18]. Intergenomic recombination events tend to maintain a largely non-clonal population structure, although clonal clusters are clearly detectable in *N. meningitidis* by multilocus sequence typing (MLST). This has delineated several meningococcal hypervirulent lineages that are responsible for epidemics worldwide[11,19].

Colonization and virulence factors

The key structures at the interface between the host and *Neisseria* spp. are the polysaccharide capsules and/or lipopolysaccharide (LPS) that may shield bacterial surfaces from the host innate and adaptive immune effector mechanisms, and the protruding surface proteins that are known as pili (hair-like projections; also known as fimbriae) (FIG. 2; TABLE 1). Pili facilitate adhesion to host tissues, further aided by the outer membrane adhesins, Opa and Opc, which are described below. At least 12 *N. meningitidis* LPS immunotypes, designated L1–L12, have been identified on a serological basis. The notable immunotypes are L3, L7 and L9, which can be sialylated, and L8, which lacks the terminal lacto-*N*-neotetraose (LNT) that is required for the addition of the sialic acid moiety[20]. *Neisseria* spp. also produce numerous secreted proteins (BOX 1).

Specificity for the host, as well as for tissues within the host, is believed to be attained primarily through adhesins. Additionally, *Neisseria* spp. possess host-specific iron acquisition mechanisms and numerous immune evasion mechanisms[21] (BOX 2). Host specificity poses a problem for developing animal models of

Natural competence
An innate ability of bacteria to acquire DNA from the local environment and to assimilate genetic information through homologous recombination.

Multilocus sequence typing
A technique used to characterize strains by their unique allelic profiles of a set of housekeeping genes.

Table 1 | **Medically important capsules of *Neisseria meningitidis***

Capsule	Structure	Prevalence*	Notable features
A	(α1–6)- *N*-acetyl-D-mannosamine-1-phosphate	• Africa • Parts of Asia	Disease-associated, non-sialic acid polymer
B	(α2–8)-*N*-acetylneuraminic acid	• Europe • Asia • North America • South America • Australia • New Zealand	Homopolymer of sialic acid; mimics structures present on neuronal cell adhesion molecules; poor immunogen
C	(α2–9)-*N*-acetylneuraminic acid	• Europe • Asia • North America • South America • Australia • Africa	Homopolymer of sialic acid; immunogenic
W135	6-D-Gal(α1–4)-*N*-acetylneuraminic acid	• Africa	Heteropolymer; contains sialic acid
Y	6-D-Glc(α1–4)-*N*-acetylneuraminic acid	• North America	Heteropolymer; contains sialic acid

*Some areas of the world have significantly lower rates of meningococcal disease; the reasons for this are not well understood. These include several parts of South America and Asia, Mexico and Japan[13].

the disease, and as a result most of our knowledge of the pathogenic mechanisms of *Neisseria* spp. comes from *in vitro* investigations.

Mechanisms of phase and antigenic variation

Aside from the gene conversion events mentioned above, other mechanisms operate in *Neisseria* spp. to give rise to phase variation and structural or antigenic variation. Phase variation occurs primarily through the process that is commonly referred to as slipped strand mispairing (SSM) and involves DNA slippage induced by repetitive sequences of nucleotides within or upstream of genes. This results in translational control of expression, which can reversibly switch gene expression on and off[4,20], or transcriptional control of expression, which can change the level of gene expression (as in the case of Opc)[22]. Antigenic variation can arise as a result of phase variation of one or more enzymes involved in LPS biosynthesis[20] or of distinct Opa proteins, as described below. In the case of PilE (pilin), the main subunit that makes up the pilus fibre, variations arise from intergenomic and intragenomic recombinase A-dependent recombination events between one of several *pilS* (silent) pilin genes and *pilE*, the expressed pilin gene[3,23].

Redundancy, antigenic and phase variation. The major adhesins (pili and Opa), which enable anchorage to host tissues, have been long recognized in *N. meningitidis* and *N. gonorrhoeae*. In addition, Opc expressed in *N. meningitidis*, but not *N. gonorrhoeae*[24], is also an important adhesin (FIG. 2). Numerous additional apparently minor adhesins (several of which were identified by homology searching of the available genomes) are generally expressed at low levels *in vitro* but may be important *in vivo*. For example, in restricted iron environments, such as might be encountered *in vivo*, the transcriptome of *N. meningitidis* is considerably altered[25] and as a result the minor adhesins may become expressed. Furthermore, several adhesins are subject to antigenic variation and/or phase variation, which can reach high frequencies and may vary between strains (see *N. gonorrhoeae* pilin variation rates[26]). Surface modulation facilitates evasion of immune effector mechanisms but can require multiplicity of adhesins (redundancy) to maintain colonization. Several adhesins may also operate simultaneously to increase the avidity of bacterial binding to the cell surface. This is often a prelude to internalization into epithelial cells[27,28], which is another immune evasion strategy.

Such constant variation renders most important surface components unsuitable as vaccine candidates. However, structures that are required for survival *in vivo* (for example, capsules, which are further described below) have been used successfully to protect against several virulent *N. meningitidis* strains. In other cases, such as in the case of the Opa protein family, the frequency of expression, abundance and functional conservation (and therefore a degree of structural conservation) suggests they might be appropriate vaccine candidates[29,30]. These observations highlight the need for in-depth studies on the structure–function relationship of members of the Opa family; understanding their modes of action, for example, their mechanisms of host-cell receptor targeting, could lead to intervention strategies to prevent infection.

Surface sialic acids, pathogenicity and modulation of adhesion. The capsule is a highly hydrated structure and is thought to protect meningococci during airborne transmission between hosts[31]. Once in the respiratory tract, meningococci may become non-encapsulated through numerous genetic mechanisms[32,33]. One of these mechanisms may involve the induction of *crgA*, a gene that is upregulated on contact with target cells and the product of which is a transcriptional regulator of several genes, including those that are involved in capsule biosynthesis[33]. Even though many bacterial isolates from the nasopharynx are non-encapsulated, disseminated infections are almost always caused by encapsulated bacteria. The capsule can prevent antibody and complement deposition[22], it is anti-opsonic and anti-phagocytic and it aids survival in the blood. Indeed, the serogroup B capsule has been shown to inhibit serum immunoglobulin G (IgG) deposition and, perhaps consequently, complement deposition on the bacteria (S. Ram, personal communication). High levels of capsule expression can also inhibit complement-mediated lysis in the presence of bactericidal antibodies that are specific for PorA or are raised against whole cells[34].

One of 13 distinct capsular structures can be expressed by meningococcal strains and form the basis for the classification of *N. meningitidis* into different serogroups. Disease is caused most frequently by strains of serogroups A, B and C, followed by W135 and Y; the other serogroups rarely cause disease[1]. Four of these serogroup capsules contain sialic acid (TABLE 1), which is important for immune evasion[35]. As they are the outer-most structures of the bacterium and because of their importance in disseminated

Phase variation
Reversible switching on and off of surface antigens that helps bacterial evasion of host immune responses.

Anti-opsonic
A term applied to agents that prevent the binding of opsonins (for example, antibodies) that enhance phagocytosis.

Serogroup
A designation denoting the immunochemistry (structure) of the capsule polysaccharides of *Neisseria meningitidis*; *N. meningitidis* strains are divided into serogroups based on the reactivity of strains with antibodies against distinct capsule structures.

Box 1 | **Secreted and translocated proteins of pathogenic *Neisseria* spp.**

Neisseria spp. have three of the six protein secretion mechanisms of Gram-negative bacteria: the autotransporter (AT) pathway, the two-partner secretion (TPS) pathway (collectively called type V secretion) and the type one secretion system (T1SS)[116]. In *Neisseria gonorrhoeae*, a type IV system is also present and seems to be devoted to DNA transport[18]. Thirteen ATs have been identified within neisserial genomes. However, *N. gonorrhoeae* contains only two of these, immunoglobulin A (IgA) protease and adhesion penetration protein (App), whereas *Neisseria meningitidis* contains several additional ATs. Those involved in adhesion are described in the adhesion section in the main text.

IgA protease

Until recently, IgA protease was the only well-recognized secreted protein of pathogenic *Neisseria* species. It contains a serine protease domain, which facilitates the release of its functional passenger domain from its cell-bound carboxy-terminal transporter domain. IgA protease is present only in pathogenic *Neisseria* species. Besides autocleavage and the cleavage of human mucosal IgA1, IgA protease can also cleave a similar site in the host lysosomal LAMP1 (lysosome-associated membrane protein 1). Although cleavage of IgA1 prolongs bacterial extracellular survival at mucosal locations, cleavage of LAMP1 may be important for the intracellular survival of the pathogens[116].

RTX-like molecules and type I secretion

Isolates of *N. meningitidis* but not *N. gonorrhoeae* secrete iron-regulated proteins termed Frp A and FrpC. These proteins are homologous to the RTX (repeat-in-toxin) family of proteins of many Gram-negative bacteria that function as cytolysins or proteases. However, their function in *N. meningitidis* remains unclear. The Frp proteins do not seem to be essential for disease but are expressed during disease and may have a role in bacterial dissemination. The exoproteins have been shown to be secreted through a functional T1SS, which is homologous to the *Escherichia coli* haemolysin system, but comprises genes that are scattered within the meningococcal genome[116].

Translocation of porins

The trimeric porin proteins, the most abundant proteins of *Neisseria* spp., form hydrophilic nutrient and ion channels in the outer membrane. They are highly immunogenic, which makes them important serological markers and potential protective antigens. An interesting property of *N. gonorrhoeae* PorB is its ability to translocate from live bacteria into eukaryotic cells and localize into mitochondria, where it interacts with the voltage-dependent anionic channel, thereby modulating apoptosis[117]. Porins may also assist in cellular invasion[118] and have been shown to act cooperatively with pili to modulate cell signalling through sequential induction of Ca^{2+} flux into and mobilization of intracellular Ca^{2+} within the target cells[119].

infections, capsules are good vaccine candidates. Current capsule-based vaccines against *N. meningitidis* target specific serogroups (A, C, Y and W135). However, serogroup B remains a problem for vaccine design, as it is not an effective immunogen owing to its structural similarities with glycans on human neuronal-cell adhesion molecules (TABLE 1).

Sialic acids are also present on the LPS of both *N. meningitidis* and *N. gonorrhoeae*. *In vitro* studies have shown that the addition of sialic acids to LPS can impart capsule-like properties to LPS, making the bacteria more resistant to antibody and complement-mediated killing and more able to avoid phagocytosis. Both LNT and sialylated LPS also mimic host-cell surface structures, which facilitates avoidance of the host antibody response[2,35–37]. However, (α2,3)-linked sialic acid on the LPS of *N. meningitidis* is recognized by sialic-acid-binding immunoglobulin-like lectins (Siglecs), which are present on some phagocytic cells. Thus, the expression of LPS sialic acids can potentially render bacteria more susceptible to phagocytosis[38]; the *in vivo* importance of this is not known. *N. gonorrhoeae* phenotypes that have unsialylated LPS use another host receptor, the asialoglycoprotein receptor (ASGPR), to interact with host urethral epithelial cells[2].

Meningococcal LPS is responsible for eliciting inflammation during sepsis[39] and is also highly toxic for human endothelial cells *in vitro*. This property is augmented in the presence of pili, suggesting that the two components cooperate in signalling to endothelial cells[40]. Interestingly, co-signalling of human endothelial cells by the pili and LPS of *N. meningitidis* was recently reported, and this results in bacterial uptake by non-phagocytic cells[41].

Neisserial strains that harbour genes for the synthesis of sialic-acid-containing capsules can generate an endogenous source of sialic acid and can add this moiety to LNT of LPS through LPS sialyl transferase, which is present in strains of both *N. meningitidis* and *N. gonorrhoeae*. However, *N. meningitidis* serogroup A strains and *N. gonorrhoeae* do not synthesize sialic acid and therefore require an exogenous source for this purpose. Indeed, during infection, they acquire sialic acid from host fluids[37]. Thus, the surface of *N. meningitidis* and *N. gonorrhoeae* can be either devoid of, or encased in, one to several layers of negatively charged molecules that are provided by the capsule and/or sialylated LPS.

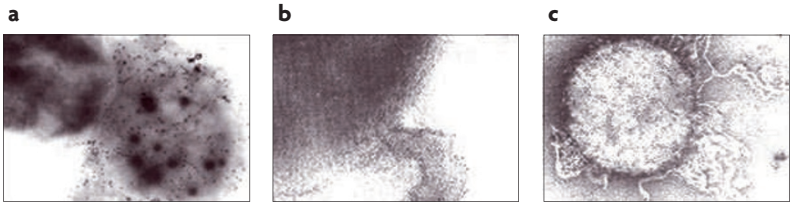
In addition to inhibiting opsonization-mediated phagocytosis and detection by complement, surface glycans can inhibit the function of non-pilus outer membrane adhesins and invasins by their juxtaposition and by charge neutralization, and thus are also anti-adhesins. As a result, non-encapsulated bacteria and those lacking sialic acids on LPS are the most invasive[36,42]. However, to cause disseminated disease, *N. meningitidis* requires the protection that is provided by surface sialic acids, especially those that are present in the capsule[13,32]. Therefore, one possible sequence of events during dissemination from the site of colonization is that capsule expression is switched from on to off and then on again to survive in the blood (BOX 3).

Major adhesins of pathogenic *Neisseria* spp.

Of all the putative adhesins that have been identified so far, pili, Opa and Opc are expressed in the greatest abundance. Comparison of *in vitro* observations shows important quantitative differences between the interactions that are mediated by the major adhesins and several newly identified minor adhesins. The major adhesins also have the tendency to enhance bacterial self-agglutination, a phenomenon that influences bacterial adhesion levels.

Pili, the polymeric pericellular glycoproteins. Recent systematic genetic analyses have identified 15 proteins that are involved in the biogenesis, assembly and disassembly of pili (known as Pil proteins), and have begun to assign precise roles to them[43]. The pilus fibre consists of numerous PilE (major pilin) subunits arranged in a helical configuration. In addition, several minor subunits (PilC, PilV and PilX) can

Box 2 | **Immune evasion**



Numerous distinct mechanisms enable bacteria to avoid both cellular and humoral immunity. These can be grouped broadly into three strategies. The first, avoidance of immune effector mechanisms, helps reduce the recognition of bacterial surface structures and includes antigenic and phase variation (see the figure part **a**, an electron micrograph showing the expression of Opc protein detected using immunogold-labelled Opc-specific antibody in one of a pair of diplococci) as well as molecular mimicry (exemplified by serogroup B capsule, which, by mimicry of glycans on host neuronal-cell adhesion molecules, avoids recognition as a foreign antigen). In addition, *Neisseria* spp. may enter epithelial and endothelial cells, which physically shields them from professional phagocytes, antibodies, complement and antimicrobial peptides.

The second strategy, diversion, involves the production of excess outer membrane (OM), which is released as vesicles and diverts antibodies and complement away from the bacterial surface (see the figure part **b**, in which pilins stained specifically with the immunogold-labelled monoclonal antibody SM1 seem to be concentrated into extruded OM. Whereas, as shown in part **c**, other antigens such as Opc are presented equally on the bacterial surface and in extruded OM). In addition, the bacteria can produce blocking antigens, such as the reduction modifiable protein (Rmp), which lead to complement depletion without bacterial lysis, perhaps by diverting complement to sites where its effect is negligible[120]. As OM vesicle vaccines that contain Rmp have been successfully used to control local outbreaks of *N. meningitidis* infections, the importance of this phenomenon *in vivo* is unclear[121].

The third strategy is the control of immune effectors, whereby the pathogens usurp host-cell signalling mechanisms such that antimicrobial mechanisms are inhibited[122]. Downmodulation of the complement cascade is achieved through binding to complement regulatory proteins, including factor H and complement component 4 binding protein; lipopolysaccharde, sialic acid, porin proteins and the surface expressed lipoprotein GNA1870 may be involved in a species-specific manner[35,123]. Recently, binding to vitronectin, an inhibitor of the membrane attack complex, was demonstrated in Opc-expressing *N. meningitidis* and resulted in increased serum resistance of Opc-expressing phenotypes (M.V. and N. J. Griffiths, unpublished observations). Images **b** and **c** are reproduced, with permission, from REF. 67 © (1992) John Wiley & Sons.

be incorporated in the fibre and modulate its function. Neisserial pilins undergo several distinct post-translational modifications, such as glycosylation[44,45] (reviewed in REF. 46), which can indirectly have an effect on cellular interactions, perhaps by affecting the agglutination of pili[44,47]. It has also been suggested that in *N. meningitidis* glycosylation is required to produce S pilins, which are truncated soluble pilin subunits that are not assembled but are secreted and could have a function in immune diversion and/or adhesion[48]. In *N. meningitidis*, the *pgl* (6-phosphogluconolactonase) gene cluster controls the modification of pili with glycans, the structure of which is determined by polymorphisms in pilin glycosylation genes and phase variation of glycosylation enzymes[49–51]. Whether this property has a role in increasing bacterial pathogenic potential is unsubstantiated.

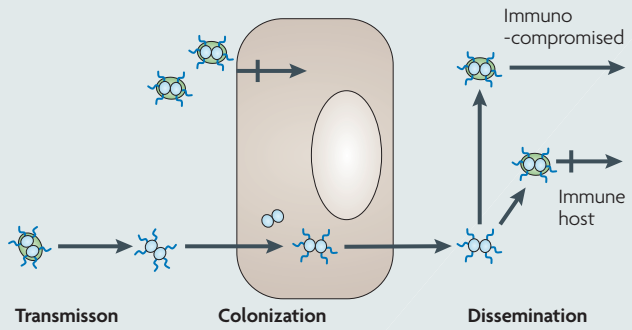
Several modifications of pilin at serine 68 have been described. This residue may be modified with phosphoethanolamine or phosphorylcholine, or may remain unmodified[52,53]. These modifications alter the charge at this position, thus potentially affecting cellular interactions and immune recognition. Phosphorylcholine has been proposed to be a potential broadly effective vaccine candidate, as it is present on surface components of many mucosal organisms. The roles attributed to phosphorylcholine in various pathogens include mimicry of platelet activating factor (PAF), enabling binding to host cells through the PAF receptor and neutralizing the functions of host cationic antimicrobial peptides[54]. Interestingly, in commensal *Neisseria* spp. phosphorylcholine occurs on LPS and not on pili. In this case, incorporation of phosphorylcholine seems to involve the *lic1* locus, which resembles that of *Haemophilus influenzae* and is absent from pathogenic *Neisseria* species[55,56]. In pathogenic *Neisseria* spp., phosphoethanolamine and phosphorylcholine can be incorporated by a single pilin phospho-form transferase enzyme (PptA). Moreover, PilV negatively modulates pilus modification by reducing the addition of both phosphoethanolamine and phosphorylcholine to pili in an as yet unresolved manner[52,57,58].

The structure of the *N. gonorrhoeae* pilin was determined in 1995 (REF. 45), and a new high resolution structure has been derived recently by a combination of the crystal structure of the pilin subunit and a three-dimensional cryo–electron microscopy reconstruction of the pilus filament[59]. In the assembled pili, the variable domains and the post-translational modifications are exposed on the surface (FIG. 2). Furthermore, the pilus contains alternative patches of positively and negatively charged regions. Glycans and phosphoethanolamine lie within negative patches in the assembled pili, and therefore their variations (phase or antigenic) are likely to modulate the adhesion properties of the pilus[59].

It thus seems that pili maintain little structural conservation to allow host immune recognition, and past vaccine trials (in which the vaccine was based on the pilus structure) showed no protection against heterologous challenge[60]. However, minor conserved pilins, such as PilX, that function indirectly to increase host-cell targeting by increasing adhesion between bacteria might be useful vaccine antigens[61].

In addition to adhesion, pili are involved in several other functions. For example, they facilitate uptake of foreign DNA from the extracellular milieu, thereby increasing the transformation frequency of bacteria and maintaining the genetic diversity that underpins the success of *Neisseria* spp. in the human host[62]. The pili of both *N. gonorrhoeae* and *N. meningitidis* are dynamic, as they can assemble and disassemble rapidly, and this is facilitated by the coordinate action of PilC and the ATPase PilT, resulting in 'twitching motility' (REF. 65). By this process, a level of movement on cell surfaces of around 1 μm per second may be attained[64]. Extension and cellular attachment followed by retraction or disassembly of the pili may decrease the distance between the bacterial and eukaryotic membranes, thereby enabling the uptake of DNA and intimate cellular interactions through integral outer membrane adhesins. The considerable mechanical force that is generated by the process of pilus retraction may also be responsible

Box 3 | **Modulation of surface glycans and pathogenesis: a possible scenario**



The capsule and lacto-*N*-neotetraose (LNT) of lipopolysaccharide (LPS) are spontaneously phase variable in *Neisseria meningitidis* at high frequencies (10^{-3}–10^{-4} per generation). During colonization, a non-encapsulated, non-sialylated phenotype is frequently present. The loss of capsulation and of sialic acid on LPS may help to establish long-term nasopharyngeal carriage by becoming intracellular, thereby avoiding host complement and phagocytic defences. In addition to genetically programmed spontaneous phase variation, environmental factors and host-cell contact may also regulate capsule expression. Dissemination from the site of colonization would then require upregulation of the generation of the capsule, as non-encapsulated bacteria are unlikely to survive in the blood. Alternatively, because the blood provides an environment in which meningococci can grow rapidly, it is possible that a small number of encapsulated organisms, arising as a result of natural phase variation or induced by environmental factors, would be selected for in the blood.

The invariable association of the capsule with disease and its role in protection from desiccation, as well as the high frequency of isolation of non-encapsulated bacteria from the nasopharynx, suggest a lifestyle requiring phenotypic transitions: sialylated (transmission phenotype) to non-sialylated (colonizing phenotype) and finally back to sialylated (transmitting and disseminating phenotype). In this model outer membrane adhesins and invasins may contribute greatly to host invasion.

for numerous signal transduction events, including the formation of cortical plaque structures and the shedding of the complement regulatory factor CD46, mediated by unknown cell-surface receptors[63,65].

The opacity proteins. The Opa proteins of *Neisseria* spp. (initially termed PII or class 5 proteins) impart opacity to colonies that express the proteins[66]. *N. meningitidis* has an additional opacity protein, Opc. In *N. meningitidis*, colony opacity can only be seen clearly in non-encapsulated bacteria[67] (FIG. 3). Opa proteins are a family of related transmembrane molecules that form eight-stranded β-barrel structures in the outer membrane of the bacterium with four surface-exposed loops (FIG. 2). Extensive structural variation occurs both within and between *N. meningitidis* strains in three of these loops. Furthermore, a single strain may express one to several Opa proteins, and alternate phase on/off of distinct *opa* genes can also give rise to antigenic variation. In addition, homologous recombination can increase the repertoire of Opa structures in a population[4,68]. Immunodominant regions of Opa proteins are contained within the variable regions of the protein, and as bactericidal antibodies elicited in the host are specific for the bacterial Opa type, their efficacy as cross-protective antigens is limited[69]. Adhesion-blocking antibodies have been generated by immunisation with purified Opa proteins, but these had no significant bactericidal or opsonic activity[70]. However, it seems that specific sets of Opa variants are prevalent in *N. meningitidis* isolates[30]. Besides immunological selection, such a repertoire of Opa proteins could arise as a consequence of functional constraints, perhaps directed by their ability to bind to carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1; discussed below). This suggests that Opa proteins could serve as potential vaccine components[30].

N. meningitidis Opc (OpcA) is encoded by a single gene and does not vary greatly in structure[22,24]. Expression of Opc has not been shown in *N. gonorrhoeae*, and in fact certain *N. meningitidis* clonal lineages, such as ET37 complex, lack *opcA*[71] and tend to cause severe sepsis instead of meningitis[72–74]. These observations have led to the speculation that Opc might be important in *N. meningitidis*-induced meningitis[72].

Newly identified adhesins

Several minor adhesins or adhesin-like proteins have recently been described as a result of genome mining for the identification of new vaccine candidates. Their properties are summarized in TABLE 2.

NhhA and App. Both *Neisseria* hia homologue A (NhhA) and adhesion penetration protein (App) resemble the *H. influenzae* autotransporter proteins Hsf/Hia and Hap, respectively. NhhA is found in most disease-causing *N. meningitidis* isolates, but is absent from *N. gonorrhoeae*[75,76]. It mediates low levels of adhesion to epithelial cells, to heparan sulphate proteoglycans (HSPGs) and to laminin[76]. App is present in all neisserial genomes that have been sequenced, including commensal *Neisseria* species. It has been implicated in regulating interactions between the bacteria and the host tissue by mediating adhesion during the early stages of colonization, before it is autocleaved. At later stages, App autocleavage may allow bacterial detachment, therefore facilitating bacterial spread[77].

HrpA–HrpB system. Recently, a two-partner secretion system, haemagglutinin/haemolysin-related protein A (HrpA)–HrpB, has been found in all strains of *N. meningitidis*. HrpA is the secreted effector protein and HrpB is the transporter component. A small proportion of HrpA remains associated with the outer membrane of *N. meningitidis* and according to one study contributes to bacterial adhesion to some epithelial cell lines[78,126].

NadA and MspA. Neisserial adhesin A (NadA) belongs to the oligomeric coiled-coil (Oca) family of adhesins and seems to be more commonly associated with disease isolates than with carriage isolates and can mediate cellular adhesion[79]. Different alleles of the gene are found in three out of four hyper-virulent *N. meningitidis* lineages, but is largely absent from carrier strains and is not found in *N. gonorrhoeae*. The level of NadA expression may vary with the phase of bacterial growth and by SSM, as NadA contains tetra-nucleotide repeats (TAAA) in its promoter

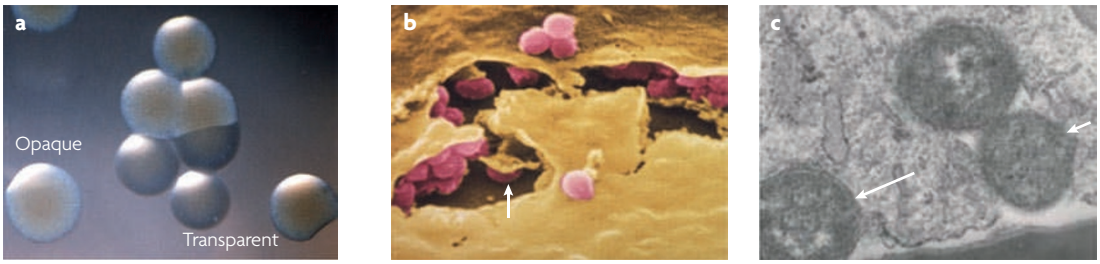


Figure 3 | **Characteristics and invasion mechanisms of *N. meningitidis* Opc protein. a** | Colony opacities of Opc phase variants of *Neisseria meningitidis*. Colonies of *N. meningitidis* expressing Opc (or Opa) generally appear opaque when viewed using oblique substage lighting, whereas Opc-deficient bacteria give rise to transparent colonies. **b** | Opc interacts with serum factors to target integrins that recognize an Arg–Gly–Asp motif, and this results in efficient invasion of host cells, as can be seen in the scanning electron microscopy (EM) image of a fractured endothelial cell with a large number of intracellular *N. meningitidis* (indicated by the arrow). **c** | This also leads to transcytosis of *N. meningitidis*, as shown by the transmission EM image, in which a cross-section of a human endothelial cell with invading *N. meningitidis* (indicated by arrows) emerging from its basolateral surface is depicted. Part **c** is reproduced, with permission, from REF. 67 © (1992) John Willey & Sons.

region[80]. NadA is expressed in several hyper-virulent lineages and is a proposed vaccine candidate against serogroup B *N. meningitidis* because it induces protective immune responses[81].

Meningococcal serine protease A (MspA) is also expressed by several but not all virulent *Neisseria* strains. It is reported to mediate binding to both epithelial and endothelial cells and to elicit the production of bactericidal antibodies[82].

Adhesion receptors and targeting mechanisms

Pili. As the capsule may protect *N. meningitidis* from desiccation during transmission between hosts, the organisms that are first encountered by the host are likely to be encapsulated. In such encapsulated organisms, the juxtaposition of the capsule masks many of the non-pilus adhesins and can render them functionally ineffective[42,67,83]. Therefore, pili are thought to have a crucial role in the initial establishment of encapsulated *N. meningitidis* on mucosal surfaces, facilitating penetration of the negatively charged barrier at the host–pathogen interface[84]. However, their targeted sites on mucosal surfaces are not random. For example, the pili of *N. meningitidis* interact with non-ciliated cells of the respiratory epithelium but do not interact with ciliated respiratory cells[85].

In general, *in vitro* studies show little binding of *N. meningitidis* or *N. gonorrhoeae* pili to non-human cells[44,86]. Pili are also thought to be primary determinants of specificity for human epithelial and endothelial cells[44,87] and those of *N. meningitidis* are known to mediate adhesion to cells of the human meninges[88]. In addition to this, binding to various other human cells has been demonstrated, including colonic cells[86] and erythrocytes; in the case of erythrocytes, attachment is thought to be mediated primarily by PilE[85,89].

Pilus receptors. Binding of the pili of *N. meningitidis* and *N. gonorrhoeae* to host cells is thought to involve CD46[90], but not all studies support this observation[87,91]. *N. gonorrhoeae* pili may also bind to complement component C4 binding protein (C4BP) and complement receptor 3 (CR3; also known as αM-integrin). Binding to C4BP could be important for serum resistance, and binding to CR3 aids colonization of the cervical epithelium with the help of porins[2,92].

Opacity protein receptors. As basic proteins, Opa and Opc use some common host-cell receptors when they target negatively charged structures on host-cell surfaces; these include HSPG and sialic acids[93,94]. The interaction between opacity proteins and sialic acids on LPS (which is also possible) can interfere with the recognition of target-cell receptors, which explains why there are reduced host–bacterium interactions following LPS sialylation[94]. HSPGs are targeted by a few *N. meningitidis* Opa proteins and several *N. meningitidis* Opa proteins tested[93,95,96]. HSPGs can also be used as receptors by the *N. meningitidis* Opc protein[97,98]. Opc may also directly bind to extracellular matrix (ECM) proteins, such as vitronectin and fibronectin[72,99]. As HSPGs interact with many ECM proteins, binding to HS molecules or ECM proteins introduces a complex array of molecular interactions between bacteria and the target cell[100]. Opc interactions with serum factors such as vitronectin and fibronectin leads to bacterial binding to endothelial αVβ3-integrin (the vitronectin receptor) and α5β1-integrin (the fibronectin receptor)[42,72,99]. This seems to be the main mode of interaction between Opc and polarized human endothelial cells (FIG. 3). Once inside the cells, *N. meningitidis* could escape the phagocytic vacuole[126] and has been shown to bind to intracellular alpha-actinin through the Opc protein[127].

In addition to the targets mentioned above, >90% of isolates of *N. meningitidis* and *N. gonorrhoeae* were shown to bind to CEACAM1. CEACAMs belong to the immunoglobulin superfamily[29] (FIG. 4) and include several members (for example, CEACAM1, CEA and CEACAM3) of which CEACAM1 is the most widely distributed[101]. The binding sites of Opa proteins reside on the amino-terminal domains of the CEACAM family, which are largely conserved and therefore allow one or more Opa proteins to target several distinct

Meninges
Membranes that envelop the central nervous system.

Table 2 | **Adhesion and invasion proteins**

Surface structure	Properties	Species	Features	Function	Target	Receptors
Pili (type IV; made up of pilins)	15–20 kDa	*Neisseria meningitidis* and *Neisseria gonorrhoeae*	Polymeric extended fibres; phase and antigenic variation	Adhesion and invasion	Human epithelial cells, endothelial cells and RBC	CR3 and CD46?[87,90,91]
Opa	24–35 kDa; heat modifiable	*N. meningitidis* and *N. gonorrhoeae*	Eight-stranded β-barrel; phase and antigenic variation	Adhesion and invasion	Human epithelial cells and fibronectin	CEACAMs, HSPG, ECM and integrins
Opc	24–35 kDa; heat modifiable	*N. meningitidis*; absent in a few lineage	Ten-stranded β-barrel; phase variation	Adhesion and invasion	Human epithelial cells, endothelial cells, activated vitronectin and fibronectin	ECM, integrins and HSPG
NhhA	57 kDa	*N. meningitidis*	Trimeric autotransporter; *Haemophilus influenzae* Hsf/Hia homologue	Adhesion	Human epithelial cells	Laminin and HSPG
App	160 kDa	*N. meningitidis* and *N. gonorrhoeae*	Autotransporter; *H. influenzae* Hap homologue	Adhesion and spread	Human epithelial cells	Protein; identity not known
HrpA (TPS)	180 kDa	*N. meningitidis*	*Bordetella pertussis* FHA homologue	Adhesion?[78,126]	Some human epithelial cell lines	Unknown
NadA	38 kDa	Expressed by some lineages of *N. meningitidis*	Autotransporter of Oca family; phase variation	Adhesion and invasion	Human epithelial cells	Protein; identity not known
MspA	157 kDa	Expressed by some lineages of *N. meningitidis*	Autotransporter; secreted	Adhesion	Human epithelial and endothelial cells (when expressed by *Escherichia coli*)	Unknown

App, adhesion penetration protein; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CR3, complement receptor 3; ECM, extracellular matrix; HSPG, heparan sulphate proteoglycan; HrpA, haemagglutinin/haemolysin-related protein A; MspA, meningococcal serine protease; NadA, neisserial adhesin A; NhhA, *Neisseria* hia homologue A; Oca, oligomeric coiled-coil; RBC, red blood cell; RecA, recombinase A; TPS, two-partner secretion.

CEACAMs[29,102,103]. As CEACAMs may contain immunoreceptor tyrosine-based inhibitory motifs (ITIMs) or immunoreceptor tyrosine-based activation motifs (ITAMs)[101], the consequences of downstream signalling following bacterial ligation depend on the receptor and target cell involved. From studies so far, it can be concluded that Opa–CEACAM interactions result in cellular invasion[28,96,102].

Overall, it seems that tissue tropism may be influenced by pili, whereas host specificity may be determined by pili as well as Opa, as both seem to bind only to human receptors. As discussed above, the main receptors for the opacity proteins are known, but the identity of pilus receptors remains unclear. Further studies are needed to clarify the nature of molecules that are targeted by pili, especially in the case of *N. meningitidis*, as this will facilitate the generation of appropriate transgenic animal models of the disease. Future studies also need to use primary respiratory epithelial cells to assess the importance of identified receptors and indeed their precise distribution and levels of expression at various sites during health and disease. Another area of potential host-cell entry, the M cells of nasal epithelium-associated lymphoid tissue[104], have also not been studied in detail for their role in neisserial transport.

Host susceptibility

Aside from the bactericidal capacity of the host, several other factors may contribute to increased host susceptibility to meningococcal infection, and may include several genetic polymorphisms[105]. Epidemiological studies also suggest that other compromising factors may contribute to host susceptibility, including physical damage to the mucosa that may ensue during respiratory infections (for example, viral infections in the winter months in the United Kingdom), dry atmospheric conditions (for example, in dry seasons in Africa) and smoking[1]. In addition, in several studies, synergism between specific viral and bacterial infections has been observed[1,106]. As meningococcal infection is not concurrent but follows influenza virus infection after a lag period[1], it would seem that changes induced by viral infection other than physical damage could account for increased *N. meningitidis* infections. In this context, remodelling of mucosal

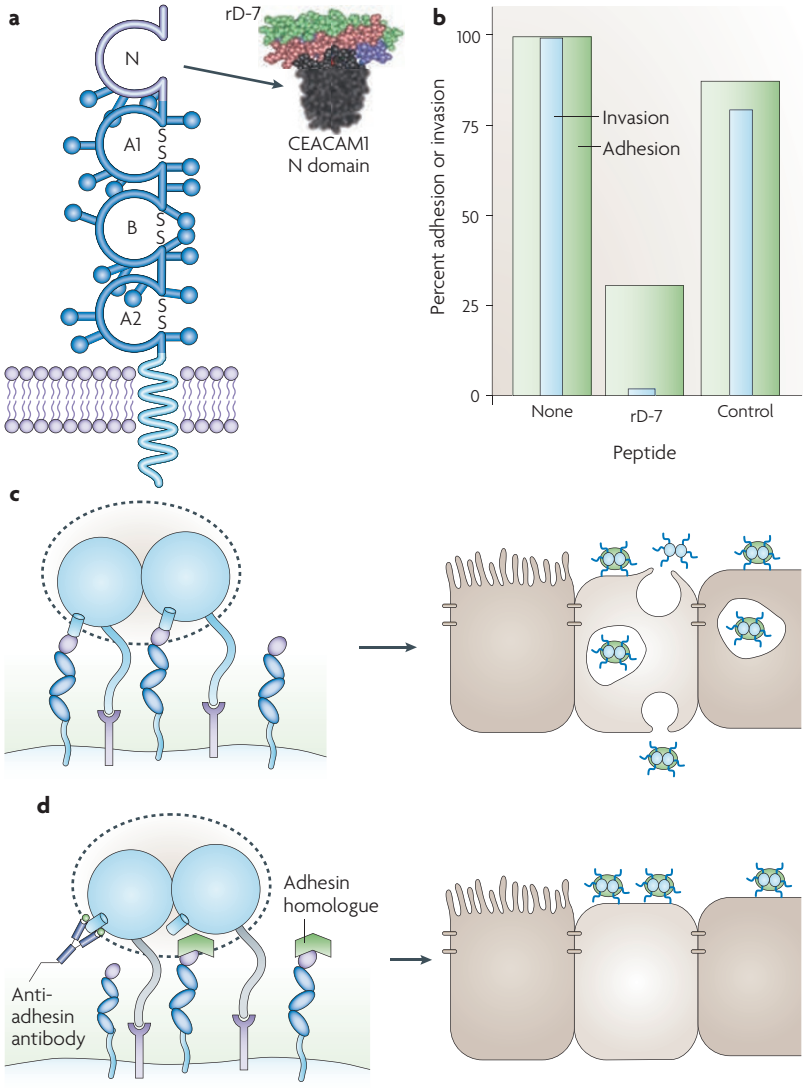


Figure 4 | **CEACAM1 as a common receptor of respiratory bacteria and a potential target for intervention.** Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1; see the carcinoembryonic antigen homepage) is the main receptor targeted by *Neisseria meningitidis* Opa proteins. It belongs to the immunoglobulin (Ig) superfamily and contains an amino-terminal Ig variable (IgV)-like domain that is targeted by *N. meningitidis* Opa, *Haemophilus influenzae* P5 and *Moraxella catarrhalis* UspA1 adhesins. A recombinant trimeric coiled-coil molecule (rD-7) has been derived based on the CEACAM1-binding region of UspA1. The recombinant molecule docks on to the receptor such that it blocks the binding of CEACAM1-binding bacteria as shown in the schematic (**a**). rD-7 binding to human epithelial cells that express high levels of CEACAM1 *in vitro* prevents host-cell invasion of *N. meningitidis* expressing Opa and pili that is mediated by the Opa–CEACAM1 pathway (**b**), but does not abrogate the binding of *N. meningitidis* through pili[28] as depicted in **c** and **d**. As Opa and pilus expression is common during colonization and disease, such interference either through the use of adhesin homologues or anti-adhesin antibodies (**d**) may prevent the crucial step of barrier penetration without eliminating localization of bacteria on the cell surface. The structural model showing rD-7 engaged with CEACAM1 was provided by R. B. Sessions and R. L. Brady, University of Bristol, UK.

tissues through upregulation of epithelial receptors by virus-induced cytokines might be potential determinants of enhanced bacterial adhesion and host-cell invasion.

CEACAM-density-dependent modulation of invasion. CEACAM1 can be upregulated in response to inflammatory cytokines[28,102,107]. This upregulation seems to increase Opa-mediated binding and invasion of fully encapsulated bacteria to human epithelial cells, a phenomenon that is aided by pili[28]. This is in contrast to observations on unstimulated cells that have low receptor density. It is possible that after initial host-cell–bacterium interactions through pili, bacterial and host-cell membranes are in close enough proximity for Opa and CEACAMs to engage, but further intimate interactions are inhibited by the capsule at low receptor expression levels. When receptor density is high, which would increase the functional affinity of the Opa–CEACAM interaction, such inhibition may be overcome. This provides a possible scenario in which hosts are rendered susceptible to invasion by virulent phenotypes following certain viral infections.

Natural and artificial anti-adhesion and anti-invasion measures. In contrast to the transmembrane CEACAM1, targeting of CEA, a glycosylphosphatidylinositol-anchored receptor, could lead to prevention of bacterial interactions with mucosal epithelial cells[108]. CEA is targeted by several gut pathogens through its mannosyl residues. It is shed in mg amounts daily in the gut and consequently has been proposed to form part of the innate immune response as it can act as a natural blocking agent for pathogen attachment in the gut[101,108]. Interestingly, CEA is also found in abundance on squamous epithelial cells of the tongue and oesophagus[101], and on buccal (M.V. and N.J. Griffiths, unpublished observations) and cervical epithelial cells[101]. If CEA is shed from these cells as well, its presence at these tissues may also be regarded as a host strategy to prevent neisserial adhesion to the tissues. However, fewer Opa proteins target CEA compared with CEACAM1, a property that may reflect the evolutionary arms race between the pathogen and the host.

In addition to the Opa proteins of *N. meningitidis*, two unrelated adhesins, P5 and UspA1, of the mucosal pathogens *H. influenzae* and *Moraxella catarrhalis*, respectively, have been shown to bind primarily to CEACAM1 (REFS 109,110). As their binding sites on CEACAM1 overlap, the bacteria can compete for binding to the receptor; this has been shown *in vitro*. Opa and P5 are β-barrel proteins and their binding to CEACAM1 seems to involve several regions on the proteins. UspA1 belongs to the Oca family of proteins, and a recombinant molecule (rD-7) based on the structure of UspA1 has been developed that can bind CEACAM1. Moreover, rD-7 has the ability to block the interactions of all three mucosal pathogens[111,112]. Importantly, it can significantly inhibit Opa–CEACAM1-mediated cellular invasion of the encapsulated bacteria while not eliminating pilus-mediated adhesion, which occurs through a different receptor. This also occurs in the post-inflammation models of infection in which the density of CEACAM1 on the surface of target cells is enhanced, thereby supporting high levels of cellular invasion[28] (FIG. 4).

Challenges for infection control

Meningococci excel at host adaptation. Their many adaptation mechanisms to the changing host environment pose a sizeable problem in the quest for a vaccine that will not become redundant as the bacterium develops new mechanisms to avoid host immunity. Notably, capsule switching between *N. meningitidis* of distinct serogroups has been observed in the course of natural colonization and in vaccinated individuals[113]. It is generally accepted that future successful vaccines will comprise several bacterial antigens. Such vaccines have been developed to cover the repertoire of circulating virulent strains of the bacteria[8] and are needed to guard against the emergence of new resistant phenotypes. One clear strategy for lasting protection would be to reduce or eliminate the reservoir of the bacterium from the human population. To this end, vaccines that eliminate adhesion and induce herd immunity would be particularly beneficial. However, elimination of normal commensals may encourage other more aggressive pathogens to colonize the host. Is this a serious problem for a commensal that is transient and has a low to moderate carriage rate? Although this remains to be fully evaluated, herd immunity is a notable factor in the reduction of serogroup C disease in the United Kingdom following the introduction of the meningococcal serogroup C vaccine[114].

An argument in favour of maintaining a level of *N. meningitidis* carriage is the notion that carriage is itself an immunizing event that helps to maintain long-term immunological memory. In this context, other choices may be available for infection control as specific blocking of certain interactions between adhesins and host-cell receptors at the mucosa could prevent tissue entry without eliminating carriage. The primary meningococcal invasins, Opa and Opc, and their cognate portals of cell entry, CEACAMs and integrins, could therefore be targeted specifically to interfere with the crucial step of host-cell penetration. In the case of some enteric bacteria, receptor mimics have proved effective for controlling infections (reviewed in REF. 115). However, in the respiratory tract, adhesin and receptor analogues could be challenging to administer. Furthermore, it is currently unknown whether receptor-blocking agents can be generated to specifically prevent bacterial infiltration without interfering with the physiological functions of the receptor and without any potential side effects. Alternatively, peptides corresponding to adhesion domains could be used as vaccine antigens to induce blocking antibodies. This has been shown for the recombinant molecule rD-7, as the antibodies to adhesion domain of UspA1 prevented the interaction between *M. catarrhalis* UspA1 and CEACAM1 (REF. 111). Another consideration, befitting the variable nature of the pathogen, is the possibility that other invasins that are at present not fully recognized could be upregulated *in vivo*. It is clear that more research will be required to identify any other key invasins of *N. meningitidis,* and studies so far have been hampered by the lack of a good model to study meningococcal pathogenesis.

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DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Haemophilus influenzae | *Moraxella catarrhalis* | *Neisseria gonorrhoeae* | *Neisseria lactamica* | *Neisseria meningitidis*

FURTHER INFORMATION

Mumtaz Virji's homepage: http://www.bristol.ac.uk/cellmolmed/staff/virji.htm
Sanger Institute *Neisseria lactamica* website: http://www.sanger.ac.uk/Projects/N_lactamica/
University of Oklahoma *Neisseria gonorrhoeae* Genome Sequencing Strain FA website: http://www.genome.ou.edu/gono.html
The carcinoembryonic antigen homepage: http://www.carcinoembryonic-antigen.de

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