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A bacterial siren song: intimate interactions between neutrophils and pathogenic *Neisseria*

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Preface

Neisseria gonorrhoeae and *Neisseria meningitidis* are Gram-negative bacterial pathogens that are exquisitely adapted for growth at human mucosal surfaces and for efficient transmission between hosts. One factor that is essential to neisserial pathogenesis is the interaction between the bacteria and neutrophils, which are recruited in high numbers during infection. Although this vigorous host response could simply reflect effective immune recognition of the bacteria, there is mounting evidence that in fact these obligate human pathogens manipulate the innate immune response to promote infectious processes. This Review summarizes the mechanisms used by pathogenic neisseriae to resist and modulate the antimicrobial activities of neutrophils. It also details some of the major outstanding questions about the *Neisseria*–neutrophil relationship and proposes potential benefits of this relationship for the pathogen.

Introduction

The genus *Neisseria* encompasses commensal and pathogenic species that colonize human mucosal epithelia. Commensal species, including *Neisseria lactamica* and *Neisseria mucosa*, live on the mucosal surface of the nasopharynx and rarely cause disease in healthy individuals. By contrast, the pathogenic species *Neisseria gonorrhoeae* and *Neisseria meningitidis*, both of which are closely related to commensal *Neisseria* spp., can induce inflammation and breach mucosal barriers (BOX 1). The pathogenic neisseriae are closely related and are therefore genetically and physiologically highly similar, but they differ in disease presentation: *N. gonorrhoeae* causes the prevalent sexually transmitted infection gonorrhoea, whereas *N. meningitidis* causes highly infectious meningococcal meningitis. However, they share the potential for long-term colonization of a single host in the absence of antibiotic therapy. They also both have the genetic flexibility to respond to changes within or between hosts, have similar high transmissibility between hosts and can increase the possibility of transmission by causing common asymptomatic infections that escape detection and treatment.

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Competing interests statement

The authors declare no competing financial interests.

Box 1

Neisserial Disease

Neisseria gonorrhoeae and *Neisseria meningitidis* cause an estimated 88 million cases of gonorrhoea and 500,000 cases of meningococcal meningitis, respectively, worldwide per year [4, 150]. As obligate human bacterial pathogens, they are highly evolved for colonization of human mucosal epithelial surfaces [3, 4]. Gonococci engage the epithelial surfaces encountered during sexual transmission, such as the male urethra, female cervix, the rectum and the pharynx. Whereas most urethral infections produce symptoms including dysuria and purulent discharge, infections of the cervix, rectum and pharynx are frequently asymptomatic, which complicates the diagnosis and treatment of gonorrhoea and contributes to its persistence [7]. Vertical transmission during childbirth can result in neonatal conjunctivitis.

N. meningitidis colonizes the nasopharynx of ~10% of the human population, although this can be much higher during seasonal epidemics in the African 'meningitis belt' and during other outbreaks. Only a small percentage of individuals colonized with *N. meningitidis* present with the invasive diseases of meningitis and meningococcaemia [4].

The clinical hallmark of infection by the pathogenic neisseriae is a host innate immune-driven inflammatory response, characterized by a potent neutrophil influx. The subsequent tissue damage enables bacterial access to secondary anatomical sites, which promotes much of the morbidity and mortality associated with neisserial infections. For *N. meningitidis*, these sites include the bloodstream, the skin and the meninges, and for *N. gonorrhoeae* they include the fallopian tubes, heart, skin and joints. At these sites, infection by pathogenic neisseriae has adverse outcomes, including disseminated intravascular coagulation and septic shock for *N. meningitidis*, and pelvic inflammatory disease, dermatitis, endocarditis and arthritis for *N. gonorrhoeae*.

The pathogenic neisseriae express a set of common virulence determinants that allow efficient colonization, immune evasion and transmission. Reflecting their high degree of adaptation to humans, rather than produce cytotoxins or other secreted toxic products, these bacteria have evolved specialized mechanisms to promote growth and persistence in the host (TABLE 1). *N. gonorrhoeae* and *N. meningitidis* also express species- specific factors that affect their different sites of infection, transmission and disease course [1], although many of these so-called virulence determinants are also expressed by commensal organisms [2]. Because it is difficult to separate the properties that facilitate colonization by the pathogenic neisseriae — properties which may be shared among all *Neisseria* spp. — from those that are crucial for eliciting disease, the features that define the pathogenic species remain obscure.

Given the high degree of adaptation of the pathogenic neisseriae to humans, it is noteworthy that the bacteria avoid the host adaptive immune system (BOX 2) and trigger a potent innate immune response involving neutrophils (also known as polymorphonuclear leukocytes (PMNs)). Infection stimulates the recruitment of neutrophils to the urogenital tract and cerebrospinal fluid (CSF), sites that are normally free of these cells, and the presence of copious numbers of neutrophils at sites of infection is the primary clinical manifestation of disease [3, 4]. As the first responders of the immune system to infection or injury, neutrophils possess cytoplasmic granules that contain many antimicrobial enzymes, peptides and reactive chemicals [5]. These cells either take up microorganisms by phagocytosis into phagolysosomes, which then fuse with the granules to kill the microorganisms inside, or kill microorganisms extracellularly via granule exocytosis or release of DNA-rich neutrophil extracellular traps [6]. The prevailing hypothesis emphasized in medical microbiology is that

humans are especially adept at recognizing and responding to neisserial infections, and this vigorous innate immune response accounts for much of the host damage that accompanies gonorrhoea and meningococcal meningitis (BOX 1). But, despite this potent inflammatory response, neutrophils do not clear these infections; viable bacteria can be cultured from CSF in patients with meningococcal meningitis and from gonorrhoeal secretions, in which neutrophils are plentiful. Moreover, individuals with neutrophil immunodeficiencies are not at increased risk of neisserial infection [3, 4]. These observations suggest that, instead, the recruitment of neutrophils by pathogenic neisseriae may not be especially deleterious to the bacteria and may in fact enhance the disease-causing potential of these pathogens. In this Review, we describe the interaction between the pathogenic neisseriae, *N. gonorrhoeae* and *N. meningitidis*, and neutrophils, including the recruitment of neutrophils during neisserial infections, resistance of the bacteria to the extracellular and intracellular antibacterial arsenal of neutrophils, and bacterial modulation of neutrophil phagocytosis, oxidant production and apoptosis. We propose a model whereby the interaction between pathogenic neisseriae and neutrophils promotes, rather than blocks, the infection cycle.

Box 2

Interactions of pathogenic neisseriae with the adaptive immune system

Infection by the pathogenic species *Neisseria gonorrhoeae* and *Neisseria meningitidis* is poorly controlled by the adaptive immune system owing to high-frequency antigenic variation of three major surface antigens: lipo-oligosaccharide (LOS), opacity-associated (Opa) proteins and type IV pili [59]. Variation of LOS and Opa proteins is mediated by the random switching on and off (phase variation) of accessory LOS glycosyltransferases or complete *opa* genes. Both switches occur via changes in nucleotide repeat numbers during DNA replication, altering the reading frame of the affected gene and subsequent protein production [65, 151]. Pili vary by gene conversion between silent storage copies of variant pilin information (the multiple copies of *pilS*) and the single expressed pilin gene (*pilE*) [151]; pili also phase vary through changes in nucleotide repeat number in the *pilC* genes (which maintain pilus expression on the bacterial surface) [153]. Thus, every population of pathogenic *Neisseria* spp. has subpopulations of bacteria that express antigenically distinct surface structures. It is assumed that antibodies reacting with any of these three antigens could block infection, providing the selective pressure for evolution of such complex antigenic and phase variation mechanisms. However, switching of LOS, Opa protein or pilus expression states also alters bacterial interactions with host cells such as neutrophils (see main text). *N. gonorrhoeae* has the genetic capacity to generate much more diversity in these surface antigens than *N. meningitidis*, presumably reflecting the greater chance of gonococcal transmission and reinfection. Whereas certain *N. meningitidis* capsular polysaccharides elicit a protective antibody-mediated immune response in immunized individuals — and these polysaccharides are the basis of the current quadrivalent vaccines —the presence of a capsule composed uniquely of sialic acid on serogroup B *N. meningitidis* makes this serogroup poorly immunogenic and refractory to vaccine-mediated protective immunity[4].

The pathogenic neisseriae also directly modulate adaptive immune functions. *N. gonorrhoeae* and *N. meningitidis* secrete an immunoglobulin A1 (IgA1) protease that can directly inactivate certain classes of secretory IgA in mucosal secretions [154]. Protease expression may prevent IgA-mediated opsonization of the bacteria to block phagocytosis through IgA receptors on neutrophils and other cells, and to prevent complement-mediated lysis. IgA1 protease also cleaves the human lysosome-associated membrane glycoprotein 1 (LAMP1)[155, 156], which localizes to the lysosomes of cells, including neutrophils. However, the role of IgA1 protease in infection with pathogenic neisseriae is enigmatic, as an IgA1 protease-mutant *N. gonorrhoeae* retains infectivity in the male

urethral-challenge model [157]. The pathogenic neisseriae also downregulate CD4+ T cell function [143] and kill B cells [144] in CEACAM1-dependent manners. Along with the inability of live pathogenic neisseriae to fully activate antigen-presenting cells such as dendritic cells [158], these results imply that during infections with pathogenic neisseriae there is a localized immune suppression that would contribute to long-term bacterial persistence in the host.

Initiation of infection and initial immune detection

Host cell binding

An essential first step in initiating human infection is colonization of the target mucosal epithelium (FIG. 1). The pathogenic neisseriae infect diverse mucosal surfaces, including the urethra, cervix, fallopian tubes, rectum, nasopharynx and conjunctiva [3, 4, 7]. Type IV pili mediate the initial attachment to the apical surface of epithelial cells at most mucosal surfaces, after which opacity-associated proteins (Opa proteins) drive intimate adherence and internalization [8]. In male volunteers inoculated with *N. gonorrhoeae,* only piliated bacteria efficiently colonize and produce symptomatic infection [9, 10], and the majority of the *N. gonorrhoeae* cells isolated from the male urethra and from the female genital tract during the proliferative stage of the menstrual cycle express Opa proteins [11–13]. Additional adhesins and invasins allow the pathogenic neisseriae to infect particular epithelial cell subsets. For example, lipo-oligosaccharide (LOS) with lacto-*N*-neotetraose at its terminus can bind the asialoglycoprotein receptor on urethral cells; the pilus and the porins interact cooperatively with complement receptor 3 (CR3) on cervical epithelium; and ‘moonlighting’ bacterial proteins such as ribosomal protein L12 bind the lutropin receptor on endometrial cells [14–17]. Adhesins specific to *N. meningitidis*, including outer-membrane protein C (Opc) and neisserial adhesin A (NadA), target receptors on epithelial and other cells that the bacterium encounters after penetration of the nasopharynx, including those in the bloodstream and meninges [18, 19].

Detection by the innate immune system

Like many other bacteria, pathogenic neisseriae are detected by mucosal epithelial cells and by the sentinel immune cells in the epithelium, which may include T helper 17 (TH17) cells, macrophages and dendritic cells [20–22]. These cells can detect infection or injury via the membrane-associated Toll-like receptor (TLR) and cytoplasmic NOD-like receptor (NLR) families of pattern recognition receptors [23, 24], and they therefore participate in the recognition of pathogenic neisseriae. LOS is a potent activator of the TLR4–CD14 complex, and porins and lipoprotein H.8 engage TLR2 [25–27]. Furthermore, peptidoglycan fragments in outer-membrane vesicles are recognized by NLRs in the cytoplasm of epithelial cells [28]. As a consequence, infection by pathogenic neisseriae promotes local release of interleukin-8 (IL-8), IL-6, tumour necrosis factor, IL-1β and other cytokines, creating a microenvironment that efficiently recruits and activates neutrophils [29–33] (Figure 1). Movement of neutrophils to the site of infection follows a well-described series of events, starting with increased adhesion to the vasculature, followed by transendothelial migration and crossing of subepithelial tissues and ending with entry into the infected epithelium [34]. In male volunteers experimentally infected with *N. gonorrhoeae*, neutrophil recruitment to the urethra occurred, on average, 2–3 days after infection, and the appearance of neutrophils in the urine was rapidly followed by the onset of dysuria and other symptoms consistent with acute disease [13, 35]. A similar timing of neutrophil recruitment has been observed in the mouse genital tract model of gonococcal infection [36]. By contrast, the timing of neutrophil recruitment to the meninges in response to *N. meningitidis* is less well established, but the

appearance of neutrophils in the CSF is used to help diagnose meningitis caused by *N. meningitidis* and other bacteria [4].

Survival of neisseriae after neutrophil exposure

Neutrophils have potent intracellular and extracellular antimicrobial activities. Neutrophil extracellular traps and reactive oxygen species (ROS) combat extracellular microorganisms, whereas bacteria that are taken up by neutrophils are transported into a phagosome that contains ROS, degradative enzymes and antimicrobial peptides. *In vivo*, neutrophils are closely associated with both pathogenic neisseriae: gonorrhoeal exudates and the CSF from patients with meningococcal meningitis reveal bacteria attached to and inside neutrophils. However, as these fluids contain viable bacteria, pathogenic neisseriae must possess mechanisms that allow them to survive both the intracellular and extracellular antimicrobial mechanisms of neutrophils. As described below, pathogenic neisseriae can modulate phagocytosis by neutrophils, but even those bacteria that are internalized can circumvent the intracellular antibacterial activities of the host.

Resistance to extracellular antimicrobial mechanisms

Neutrophils kill adherent microorganisms through localized granule exocytosis and extracellular production of ROS [37]. Neutrophils also produce neutrophil extracellular traps, which capture and inactivate bacteria at a distance from the neutrophil surface [6]. Despite these mechanisms, neutrophils are not especially adept at killing extracellular bacteria; when *N. gonorrhoeae* is incubated in the presence of neutrophils *in vitro*, >80% of extracellular bacteria remain viable, and neutrophils in which phagocytosis is inhibited possess a limited ability to kill *N. gonorrhoeae* [38]. At least one *N. gonorrhoeae* virulence factor, the metalloproteinase NGO1686 (which is also encoded in meningococcal genomes), protects the bacterium from extracellular killing by neutrophils [38, 39]. This observation supports the contention that *N. gonorrhoeae*, and potentially *N. meningitidis*, can withstand the extracellular antimicrobial activities of neutrophils when phagocytosis has not occurred.

Prevention of phagocytosis

The pathogenic neisseriae avoid phagocytosis by neutrophils in three ways: by preventing their binding to the neutrophil surface, by limiting the deposition of opsonic antibody or complement on the bacterial surface and by varying antigenic surface structures to evade humoral immunity (Fig. 2a).

As phagocytosis requires close apposition between the neutrophil and bacterial membranes, one effective strategy to avoid phagocytosis is to physically block this interaction. *N. meningitidis* produces a polysaccharide capsule that prevents phagocytosis by increasing the negative charge of the bacterial surface [40]. Many different capsular serotypes have been described for *N. meningitidis*, six of which are associated with epidemic disease (A, B, C, W-135, X and Y) [41]. *N. gonorrhoeae* does not produce a capsule. Interestingly, capsule expression in *N. meningitidis* can be turned on and off during the bacterial life cycle, suggesting that there are points in the life cycle at which capsule production is disadvantageous, such as when inside a host cell [41, 42]. It was originally thought that the type IV pili of pathogenic neisseriae, which extend several micrometres from the bacterial outer membrane, were antiphagocytic [43], but most research has refuted this idea [44, 45].

Efficient phagocytosis of bacteria is driven by opsonization with antibody and/or complement. Therefore, bacteria can avoid phagocytosis by preventing antibody or complement deposition. The meningococcal capsule masks bacterial surface antigens that can be recognized by antibodies, complement or lectin-type phagocytic receptors. This masking not only blocks opsonophagocytosis but also confers resistance to complement-

mediated killing [46, 47]. The pathogenic neisseriae also prevent antibody deposition by incorporating sialic acid (*N*-acetylneuraminic acid) into their surface structures. As sialic acid is also found on the surface of mammalian cells, it is not antigenic to the immune system. Because antibodies are not raised against sialylated moieties on bacterial surface structures, sialylation inhibits opsonophagocytosis of the bacteria. Sialic acid is the major component of the serogroup B *N. meningitidis* capsule and is also part of the capsules of serogroup C, W-135 and Y *N. meningitidis* [41]. Furthermore, the terminal galactose residue of LOS from pathogenic neisseriae is sialylated by the α-2,3-sialyltransferase, encoded by *lst* [48]. Reflecting the high degree of adaptation of the pathogenic neisseriae to humans, the activated sialic acid substrate for the sialyltransferase, cytidine monophospho-*N*-acetylmuramic acid, is produced by human cells, not the bacteria [49]. Bacteria with sialylated LOS have enhanced survival in the presence of human and mouse neutrophils [50] and are also more resistant to complement-mediated killing [51].

The pathogenic neisseriae also limit complement-mediated phagocytosis by binding the complement cascade inhibitors complement factor H and complement factor 4b-binding protein (C4BP). Complement factor H directly interacts with the *N. gonorrhoeae* porin PorB1A (encoded by *porB*) and sialylated LOS, and *N. meningitidis* also produces complement factor H-binding lipoprotein and neisserial surface protein A (NspA) [52–55]. C4BP binds to the PorA porin of *N. meningitidis* (encoded by *porA*) and to both PorB1A and PorB1B (also encoded by *porB*) of *N. gonorrhoeae*, contributing to bacterial serum resistance [56, 57]. The association between variants of the human complement factor H gene and serious meningococcal disease [58], and the observation that individuals lacking terminal complement components (such as C6) are more susceptible to meningococcal infection [4], underscores the importance of evading complement deposition in neisserial pathogenesis.

The pathogenic neisseriae avoid antibody-mediated opsonization by varying the antigenic surface structures. *N. gonorrhoeae* and *N. meningitidis* undergo high-frequency variation in the expression and composition of LOS, Opa proteins and type IV pili (Box 2) [59]. By continually varying the antigens that are presented to the host immune system, the bacteria stay one step ahead of the humoral immune response, thereby preventing antibody binding to the bacterial surface and subsequent phagocytosis through immunoglobulin receptors.

Phagocytosis of neisseriae

Although the pathogenic neisseriae can avoid phagocytosis in various ways, numerous bacteria are detected inside neutrophils from patients with gonorrhoea or meningococcal meningitis (in fact, the original name for *N. meningitidis* was *Diplococcus intracellularis* [4]). Experimental evidence suggests that both opsonized and non-opsonized bacteria can be phagocytosed by neutrophils (FIG. 2b). Generally, when a particle engages immunoglobulin or complement receptors on neutrophils, it is internalized into a phagosome that fuses with cytoplasmic granules. This produces an acidified phagolysosome containing ROS, degradative enzymes and antimicrobial peptides, a toxic combination [60]. Although the pathogenic neisseriae have evolved extensive mechanisms for evasion of complement and antibodies, as described above, convalescent serum from patients with meningococcal meningitis contains *Neisseria* spp.-reactive antibodies, and complement factors C3b and C4b can be fixed on the bacterial surface [4, 61]. These findings suggest that a fraction of the pathogenic neisseriae is subjected to opsonophagocytosis during infection.

In the absence of opsonization, the outer-membrane Opa proteins mediate internalization of the pathogenic neisseriae by neutrophils. *N. meningitidis* and *N. gonorrhoeae* encode up to 11 and five different Opa proteins in their genomes, respectively [62–64]; most research has focused on the gonococcal Opa proteins. Each Opa-encoding gene independently undergoes phase variation through a pentameric repeat in the DNA sequence encoding the signal

peptide, such that there are both Opa+ and Opa− bacteria in the population, and some Opa+ bacteria express multiple Opa proteins [65]. The majority of Opa proteins are recognized by one or more of the human CEACAM receptors (these Opa proteins are referred to here as Opa_{CEA} proteins), a more restricted subset binds heparan sulphate proteoglycans (referred to here as Opa_{HS} proteins) and some Opa proteins engage both types of protein [66]. *N. gonorrhoeae* expressing defined Opa_{CEA} proteins undergoes enhanced phagocytosis and killing [45, 67–70]. Moreover, heterologous expression of Opa_{CEA} proteins in *E. coli* is sufficient to promote phagocytosis by neutrophils [71], and *N. gonorrhoeae* outer membrane vesicles containing Opa proteins, or purified Opa proteins reconstituted in liposomes or detergent micelles, inhibit the interaction of homologous Opa+ bacteria with neutrophils [72]. Thus Opa_{CEA} proteins are an important mediator of neutrophil-mediated phagocytosis of pathogenic neisseriae.

The CEACAMs are members of the immunoglobulin superfamily of cell adhesion receptors and mediate intercellular associations that are important for tissue structure, neovascularization and insulin responsiveness; importantly, CEACAMs are used as receptors by non-neisserial human-specific bacterial pathogens that live at mucosal surfaces, including *Moraxella catarrhalis* and *Haemophilus influenzae* [73]. It has been proposed that CEACAM3, which is expressed only by neutrophils and other granulocytes, evolved to assist neutrophils in clearing these bacteria [74–76]. CEACAM1 and CEACAM6 are also expressed by neutrophils [77–81], and human CEACAM1, CEACAM3, CEACAM5 and CEACAM6 all bind neisserial Opa_{CEA} proteins through an interaction of their amino-terminal immunoglobulin variable-like domain with the hypervariable loops of Opa_{CEA} [82, 83]. CEACAM1, CEACAM3 and CEACAM6 possess multiple extracellular immunoglobulin-like domains, and each protein transduces different intracellular signals. Membrane-spanning isoforms of CEACAM1 have two cytoplasmic immunotyrosine inhibitory motifs (ITIMs) that block cytokine production and proliferation in T cells [84]. By contrast, transmembrane isoforms of CEACAM3 have a cytoplasmic immunotyrosine activation motif (ITAM) that stimulates kinase-dependent signalling events in neutrophils, culminating in phagocytosis, ROS production and killing of Opa_{CEA}+ *N. gonorrhoeae* [87–89]. The glycophosphatidylinositol (GPI)-anchored CEACAM6 and ‘short’ isoforms of CEACAM1 and CEACAM3 require transmembrane CEACAMs or other receptors to transduce signals [73, 84]. Intriguingly, CEACAM1 and CEACAM6 potentiate, rather than impede, CEACAM3 signalling in neutrophils infected with high numbers of *N. gonorrhoeae* [88]. This observation helps explain why *N. gonorrhoeae* that binds only CEACAM1 promotes ROS production [89] and bacterial killing [90] by neutrophils.

Opa_{CEA}+ *N. gonorrhoeae* survives less well after exposure to neutrophils *in vitro* than its Opa− counterpart [68, 69]. However, Opa+ *N. gonorrhoeae* is commonly recovered from the urethra of men with uncomplicated gonorrhoea and from the cervix of women in the proliferative phase of the menstrual cycle, two sites where neutrophils are also commonly found during neisserial infection, suggesting that at least some Opa+ bacteria survive neutrophil surveillance despite recognition by CEACAM3 [11–13]. At least two possibilities could explain the *in vivo* selection for Opa+ neisseriae despite their increased sensitivity to killing by neutrophils. The first possibility is that the selective pressure to maintain Opa expression for bacterial binding and uptake by epithelial cells outweighs the detrimental effect of Opa expression on survival after exposure to neutrophils. With this in mind, it is intriguing that a subset of neisserial Opa_{CEA} proteins are recognized by other human CEACAMs but not by CEACAM3 [79, 81]. This suggests that the pathogenic neisseriae have evolved these specific Opa proteins to maintain the capacity for mediating epithelial colonization while avoiding clearance by neutrophils through CEACAM3 [76]. As Opa expression is phase variable, a proportion of the bacterial population can avoid CEACAM3-dependent phagocytosis and killing by neutrophils, ensuring persistence of some bacteria in

the human host. The second possibility, as discussed below and suggested by *in vitro* evidence, is that not all internalized Opa+ neisseriae are killed by neutrophils. The pathogenic neisseriae may in fact exploit OpaCEA protein expression to drive their own uptake into neutrophils, which may provide the bacteria with advantages inside the host.

As well as the Opa proteins facilitating phagocytosis of non-opsonized neisseriae, adherent and chemokine-primed primary human neutrophils have been shown to internalize non-opsonized Opa− *N. gonorrhoeae* [38]. Opsonin-independent, Opa-independent phagocytosis of the pathogenic neisseriae may occur in three ways. First, neutrophils may use an unknown receptor to bind to *N. meningitidis* producing lacto-*N*-neotetraose-containing LOS [91]. Second, pathogenic neisseriae may engage CR3 through a cooperative interaction between type IV pili and the porin PorB1B, as described for the *N. gonorrhoeae* interaction with cervical epithelial cells [17]. Third, the pathogenic neisseriae may be small enough (less than 1 μm in diameter) to be internalized by receptor-independent macropinocytosis.

It is clear that the surface variability of the pathogenic neisseriae contributes to complex interactions with neutrophils that can either activate or inhibit phagocytosis and antibacterial activities. It will require systematic investigation with defined variants and mutants to determine how the expression of different bacterial surface ligands alters phagocytosis and whether these interactions dictate different fates for the bacteria within neutrophils (see below).

Neisserial survival inside neutrophils

Phagocytosis by neutrophils is generally a dead end for bacteria. However, a fraction of the pathogenic neisseriae seems to survive and replicate inside neutrophils. Electron microscopy studies revealed the presence of electron-dense, intact bacteria inside neutrophils from inflammatory exudate[44, 92–94], and through the use of membrane-impermeant antibiotics it was shown that the number of intracellular *N. gonor-rhoeae* cells and the number of bacteria per intracellular phagosome inside neutrophils increase over time [95–97]. This occurs for both serum-opsonized, Opa+ and non-opsonized, Opa− *N. gonorrhoeae*, suggesting that the route of entry does not affect the eventual replication of the bacteria inside cells [38, 98]. Although it is formally possible that the increase in bacterial numbers inside neutrophils is due to continual phagocytosis of live extracellular bacteria, and the increase in numbers of bacteria per phagosome results from fusion between phagosomes, these findings strongly suggest that *N. gonorrhoeae*, and potentially also *N. meningitidis*, survives and replicates inside neutrophils. Pathogenic neisseriae may persist inside neutrophils as a result of their intrinsic resistance to neutrophil antimicrobial factors and by interfering with the release of these factors.

Preventing PMN apoptosis

As neutrophils are terminally differentiated cells with a half-life of several hours, internalization by these cells could be considered a dead end for *Neisseria* spp., unless the lifespan of these immune cells could be increased. To this end, the pathogenic neisseriae modulate the apoptosis programmes of neutrophils and of other human cells. Experiments primarily using cultured immortalized epithelial cells have revealed both pro-and anti-apoptotic effects of pathogenic neisseriae [99–102]. Modulation of apoptosis has been linked to translocation of neisserial porin to the mitochondrion, a process which may require other factors, such as Omp85 [103]. However, the mechanism by which the translocated porin modulates apoptosis remains unknown [102, 104]. Modulation of apoptosis by a porin may also occur in neutrophils, as infection by *N. gonorrhoeae* extends the lifetime of some primary human neutrophils by delaying spontaneous apoptosis [33, 105], in contrast to phagocytosis of most bacteria, which triggers apoptosis [106]. This lengthening of the neutrophil lifespan by

pathogenic neisseriae supports the idea that these bacteria can modulate neutrophils to gain an advantage during infection of the human host.

Disarming neutrophils

Whether bacteria are inside or outside neutrophils, there are two basic mechanisms that can be invoked to explain survival of the pathogenic neisseriae among neutrophils. First, the bacteria could possess inherent resistance to neutrophil antimicrobial factors. There is strong genetic and biochemical evidence that specific gene products can resist neutrophil factors, but in many cases these protective gene products have not been directly evaluated in the context of intact neutrophils. Second, the bacteria could respond to and downregulate the antimicrobial activities of neutrophils — for example, by delaying neutrophil apoptosis and modulating release of neutrophil antimicrobial products. Given the high degree of host adaptation by the pathogenic neisseriae, it is not surprising that there is evidence that both mechanisms can act to promote bacterial survival after exposure to neutrophils.

Neisserial resistance to neutrophil-mediated oxidative damage

One of the best described antimicrobial properties of neutrophils is the oxidative burst. Activated neutrophils assemble the multisubunit NADPH oxidase enzyme on the phagosomal or plasma membrane [107] (Fig. 2c). NADPH oxidase generates superoxide, which spontaneously or catalytically dismutates to hydrogen peroxide; in turn, hydrogen peroxide is used by the neutrophil granule enzyme myeloperoxidase to produce hypochlorous acid. These ROS are microbicidal owing to their ability to damage lipids, proteins and nucleic acids [108].

To defend against ROS, the pathogenic neisseriae encode several proteins that directly detoxify these compounds (including catalase, cytochrome *c* peroxidase and superoxide dismutase) or quench them (such as the MnII transport system (MntABC)), or that repair oxidatively damaged DNA and proteins (such as MutY, RecA, the Uvr system and MsrAB) [109–117] (Fig. 2C). In addition, *N. meningitidis* counters ROS by using L-glutamate transporter (GltT) to acquire L-glutamate, which is converted to glutathione and helps maintain cytoplasmic redox potential during ROS exposure [118]. Additional proteins that protect against ROS include the gonococcal metalloproteinase NGO1686 and the proteins encoded in the NMB1436–1438 operon in *N. meningitidis* [39, 119]. In *N. gonorrhoeae* str. FA1090, transcription of many of these genes is increased following exposure to sublethal concentrations of oxidants, suggesting the presence of transcription-regulatory circuits that protect the bacteria from oxidative stress [39]. The pathogenic neisseriae also possess at least three means of inhibiting the production of ROS by neutrophils. Exposure to lactate, a by-product of neutrophil glycolysis, enhances bacterial consumption of molecular oxygen, which depletes the substrate for NADPH oxidase and thus blunts the oxidative burst [120]. Viable Opa− *N. gonorrhoeae* can also suppress ROS production through a process that requires metabolically active bacteria and direct contact with neutrophils [121]. Finally, purified porin can translocate into cells and, subsequently, into the mitochondria by a poorly understood mechanism to block ROS production [122].

Despite the presence of many bacterial antioxidant defences, there is mounting evidence that ROS have a minimal role in neutrophil killing of *Neisseria* spp. during infection. Mutants lacking antioxidant gene products, singly or in combination, survive neutrophil exposure similarly to wild-type bacteria [38, 123]. Moreover, chemical or genetic inhibition of neutrophil NADPH oxidase [38, 68, 124, 125], or incubation with neutrophils in anoxic conditions [126], does not affect bacterial survival after exposure to human or mouse neutrophils. Although some Opa+ bacteria induce ROS production by neutrophils, NADPH oxidase activity does not affect bacterial survival in neutrophils [38, 124]. Even the survival of gonococcal NGO1686

and *recN* mutants, which are significantly more sensitive to ROS and to the antibacterial activities of neutrophils than their wild-type counterparts, is unaffected by NADPH oxidase inhibition [38]. Together, these observations show that neutrophil-mediated activity against pathogenic neisseriae and the production of ROS are separable phenotypes. Notably, there are other host sources of ROS besides neutrophils. Several *N. gonorrhoeae* strains with mutations in genes encoding antioxidant proteins have survival defects in primary human cervical epithelial cells and in the mouse genital tract, implying that these cells and tissues produce ROS [126, 127]. Commensal lactobacilli that are present in the female genital tract can also generate hydrogen peroxide, although evidence from mouse models, as well as studies using human secretions, suggests that this source of ROS does not significantly affect gonococcal survival [128, 129].

One reason that the oxidative burst may be dispensable for the antibacterial activities of neutrophils against *Neisseria* spp. is that the bacteria use multiple mechanisms to defend themselves against ROS. We speculate that in early stages of infection by the pathogenic neisseriae, when few bacteria are present and most are viable, host ROS production is minimal and antioxidant proteins protect the bacteria sufficiently. However, more ROS are produced when the number of Opa+ bacteria increases and a sizable population of non-growing bacteria forms, leading to increases in the levels of inflammatory products such as LOS. We hypothesize that the ROS produced by neutrophils and other host cells acts as a signal to the bacteria that inflammation has commenced and, in response, the bacteria initiate a transcriptional programme that is protective against oxidative and non-oxidative host damage. As support for this hypothesis, pretreatment of *N. gonorrhoeae* with hydrogen peroxide enhances bacterial survival in primary human neutrophils independently of the oxidative burst [38]. Thus, the pathogenic neisseriae exploit the oxidative arsenal of the host by using it to induce the production of additional defences against neutrophils.

Neisserial resistance to neutrophil-mediated non-oxidative damage

As oxidative products are dispensable for neutrophil-mediated antimicrobial activities against pathogenic neisseriae, other, non-oxidative antibacterial factors of neutrophils must act on the bacteria, although the presence of viable neisseriae in the presence of neutrophils implies that these factors are not sufficient to clear infection. The non-oxidative antibacterial factors in neutrophils include degradative enzymes (such as lysozyme, elastase and cathepsin G), cationic peptides (such as defensins and the cathelicidin LL-37) and the vesicular proton-ATPase, which lowers the pH of the phagosome [130, 131]. Many of these factors are also present at mucosal surfaces and have important functions in protecting the host. For example, LL-37 is present in mucosal secretions, and the female genital tract has a low pH.

Many proteins of pathogenic neisseriae protect the bacteria after exposure to non-oxidative antibacterial factors *in vitro*. The multiple transferable resistance system (MtrCDE) and fatty acid resistance system (FarAB) efflux pumps play a prominent part in conferring resistance to antibiotics, free fatty acids, detergents and antimicrobial peptides [132, 133]. Gonococcal mutants lacking the MtrCDE efflux pump do not colonize the mouse genital tract as well as wild-type bacteria [134], but it is not known whether this is due to non-oxidative functions of genital neutrophils, increased sensitivity of the mutants to the antimicrobial peptides in mucosal secretions or both. The MisRS (also known as PhoPQ) two-component regulatory system, which promotes resistance to antimicrobial peptides in *Neisseria* spp. and other pathogens [135], directly and indirectly regulates various genes in *Neisseria* spp. One of the gene products in the MisRS regulon, lipopolysaccharide transporter periplasmic protein (LptA), adds a phosphoethanolamine moiety to LOS [136, 137]. Meningococcal LptA confers resistance to antimicrobial peptides as well as complement [138]. Other gene products that defend pathogenic neisseriae against non-oxidative factors include the meningococcal

proteins NMB0741 and NMB1828, which provide protection against cathelicidin [139], and peptidoglycan *O*-acyltransferase (PacA), which *O*-acetylates peptidoglycan to render it resistant to lysozyme [140]. In addition, the *N. meningitidis* capsule confers resistance to antimicrobial peptides [42].

One of the major gaps in our understanding of the interactions between neutrophils and pathogenic neisseriae is how the virulence properties that defend the bacteria against non-oxidative factors *in vitro* affect bacterial survival after neutrophil challenge. To date, only the gonococcal NGO1686 and RecN proteins have been shown to promote bacterial survival after neutrophil exposure independently of NADPH oxidase activity [38], and how these proteins mediate their effect is not currently understood. Many other proteins confer resistance to non-oxidative antimicrobial factors derived from neutrophils, but their role in protecting the bacteria from clearance by neutrophils remains unexplored.

Neutrophils may promote neisserial pathogenesis

Successful pathogens create a niche in their hosts for nutrient acquisition and a means of effective transmission to new individuals, all the while limiting deleterious effects on the health of the host. As bacteria that are well adapted to their host, the pathogenic neisseriae possess unique mechanisms to evade and subvert host immune responses. On the basis of this adaptation, we posit that these bacteria actively recruit neutrophils for intra-and interhost dissemination, in a mechanism similar to that proposed for *Staphylococcus aureus* [141]. As described above, the pathogenic neisseriae meet several criteria that would support this contention: neutrophils are recruited in unusually high numbers at sites of infection, the bacteria survive and replicate in and around neutrophils, and the bacteria use dedicated proteins to defend against killing mechanisms in neutrophils and modulate neutrophil activities. These pathogenic mechanisms have not necessarily evolved to maximize the survival of any individual bacterial cell, but probably function on a population-wide scale, enabling a subset of bacteria to undergo replication, dissemination and transmission (FIG. 3). As elaborated on below, we speculate that these pathogens actively associate with neutrophils to help themselves acquire nutrients, to create a protective niche from the immune system and to allow transmission to tissues deeper in the body and even to other hosts.

Nutrient acquisition

The pathogenic neisseriae reside primarily on the surface of epithelial cells, where the limited nutrient flow in mucosal secretions restricts bacterial replication. As part of the inflammatory response to infection by pathogenic *Neisseria* spp., neutrophil influx causes leakage of serum components and damage to surrounding tissues, both of which would liberate nutrients for extracellular bacteria. Phagocytosis of the bacteria by neutrophils may allow pathogenic neisseriae access to intracellular nutrient pools, including concentrated sources of iron, such as holotransferrin and lactoferrin, for which these bacteria have receptors[142].

Creating a protective niche

When inside neutrophils, the bacteria reside within phagosomes, which protect the pathogens from humoral immune surveillance. Neutrophils are not thought to function as antigen-presenting cells, so persistence inside neutrophils may also protect the bacteria from potentially toxic sources of cell-mediated immunity, such as cytotoxic T lymphocytes. Of course, phagocytosis of a dead, infected neutrophil by a macrophage would represent a dead end for the bacteria inside and would amplify the humoral immune response. However, infection by pathogenic neisseriae can prolong the neutrophil lifespan, and these bacteria

provide a general immune suppression to dampen adaptive immune responses [143, 144]. These two features would contribute to the persistence of the pathogens when in association with neutrophils.

Access to deeper and ascendant tissues

A small fraction of the pathogenic neisseriae transit across epithelial and endothelial monolayers, but in general these bacteria are not particularly motile; the one organelle associated with motility, the type IV pilus, in fact counters dissemination by driving the formation of bacterial microcolonies that enhance attachment to host tissues [145]. The presence of neutrophils would facilitate bacterial access to secondary sites of infection in two ways. First, the influx of neutrophils and the associated inflammatory milieu produces local tissue damage, creating breaches in epithelial integrity through which the bacteria could pass [146]. Second, as recently suggested, neutrophils themselves may carry viable bacteria to new sites [147]. Activated neutrophils could carry *N. meningitidis* throughout the nasopharyngeal epithelium or aid *N. gonorrhoeae* ascension to the upper reproductive tract. Retrograde (tissue-to-bloodstream) movement of neutrophils in sterile injury has been reported [148]; if this were to occur during infection with a pathogenic neisserial species, neutrophils could also transport the bacteria into the bloodstream to aid dissemination. Neutrophil-driven movement of neisseriae has not yet been visualized, but it would promote the main outcomes of *N. meningitidis* infections — meningococcaemia and meningitis — and would also lead to disseminated gonococcal infection and associated arthritis, endocarditis and dermatitis.

Transmission to new hosts

N. gonorrhoeae survives poorly, if at all, outside the human body, whereas capsulated *N. meningitidis* can survive for a limited duration on fomites and solid surfaces [3, 4]. It is tempting to speculate that neutrophils serve as a conduit for transfer of meningococci and gonoccoci to receptive hosts. Although there is no direct evidence for transfer of neutrophils between individuals, there is a possibility that mechanisms in addition to the transfer of free bacteria are important for the high frequencies of transmission between hosts — for instance, in the transfer of *N. gonorrhoeae* from women to men. Taking this into account, the acute inflammation associated with gonorrhoea could be a response to foreign neutrophils as well as to the bacteria[3].

Conclusions

Many crucial questions about the interactions between pathogenic neisseriae and neutrophils remain to be addressed. For instance, how do the bacteria survive and replicate in a cell type that is optimized for antibacterial function? How does the plethora of proteins that protects the bacteria from purified host-derived components *in vitro* contribute to persistence during infection? What are the mechanisms by which pathogenic neisseriae modulate neutrophil functions (ROS production, release of antimicrobial components and apoptosis)? This last question is especially intriguing considering that virulence-associated secretion systems and production of exotoxins are highly reduced or absent in these bacteria [149]. Defining the ultimate fate of the bacteria during neutrophil infection will require a system for following the entire infection process, from initial exposure to establishment of chronic infection. As ethical considerations preclude the parallel examination of *Neisseria* spp. within neutrophils in the human host, animal models, including transgenic mouse models, will probably provide the appropriate system [36]. Given the recent exciting developments in the study of the interactions between pathogenic *Neisseria* spp. and neutrophils, it is likely that advances in the genetics and genomics of *Neisseria* spp., neutrophil cell biology and *in vivo* systems

for investigating innate immune function will contribute to refining and addressing these questions in the near future.

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Glossary

Adhesins	Molecules that are expressed on a cell to mediate adherence to another surface
Apoptosis	Programmed cell death of host cells. The rate and extent of apoptosis in neutrophils and other cells can be influenced by bacterial infection
Capsule	The polysaccharide coating found on *N. meningitidis* that alters the bacterial cell surface and its interactions with other cells and molecules
CEACAM (Carcinoembryonic antigen-related cell adhesion molecule) receptors	A family of host proteins that are expressed on a variety of cell types to mediate intercell communication. They serve as receptors for opacity-associated (Opa) proteins
Commensal	Describing the relationship between two organisms in which one organism benefits while the other is unaffected
Complement	A system of >25 proteins that recognize foreign objects and target them for destruction or phagocytosis. The complement proteins undergo sequential proteolytic events, resulting in activation of the membrane attack complex, a pore that forms on the surface of the foreign object to rupture it. Complement proteins such as C3b and C4b are recognized by complement receptors, and this triggers phagocytosis of the complement-opsonized particle
Cytokines	Secreted proteins that are produced by cells of the immune system and by non-immune cells that can recognize infection, injury or inflammation. Chemokines are a class of chemotactic cytokines which recruit immune cells to specific locations in the body
Dysuria	Pain while urinating. Common in men with acute gonorrhoea
Granulocytes	Innate immune cells with a cytoplasm that is morphologically distinguished by the presence of granules. Neutrophils, eosinophils and basophils are all granulocytes
Lipo-oligosaccharide (LOS)	The main component of the outer leaflet of the outer membrane of Gram-negative bacteria. LOS is similar to lipopolysaccharide (LPS) but lacks the long sugar chain (the O antigen). Pathogenic neisseriae produce different LOS isotypes owing to phase variation of glucosyltransferases for LOS

Neutrophil extracellular traps (NETs)	Chromatin-based structures that are coated with granule proteins and are released from neutrophils to entrap microorganisms. There is currently some debate about whether neutrophil extracellular traps are released from dying neutrophils only, or whether live neutrophils can also produce them
Opacity-associated proteins	A family of proteins expressed on the surface of pathogenic neisseriae. The assortment of Opa proteins expressed by the bacteria constantly changes owing to phase variation of the *opa* genes. Opa proteins mediate bacterium–bacterium and bacterium–host cell interactions, and some alter the characteristics of neisserial colonies grown on solid media
Opsonic	Coating a particle to facilitate its uptake by phagocytes. Common opsonins are immunoglobulins (antibodies) and complement
Oxidative burst	The production of reactive oxygen species (ROS) through the action of the NADPH oxidase enzyme
Pattern recognition receptors	A family of proteins that recognize families of foreign objects, including evolutionarily conserved products of microorganisms, such as lipopolysaccharide and peptidoglycan. Common pattern recognition receptors include proteins of the Toll-like receptor family and the NOD-like receptor family
Phagocytosis	A crucial process by which the immune system clears foreign objects, invading microorganisms and dead or dying cells. Phagocytic cells of the immune system include neutrophils and macrophages; dendritic cells can also undergo phagocytosis
Phagolysosomes	Intracellular compartments that are capable of degrading material ingested by phagocytic cells. In neutrophils, the phagolysosome is formed when the early phagosome fuses with granules that carry antimicrobial products
Pilus	A fibre that extends from the bacterial cell surface to mediate adherence to a host cell
Porin	Bacterial outer-membrane, channel-forming proteins. Neisserial porins have been reported to translocate into human cells and affect mitochondrial membrane potential, cell lifespan and the neutrophil-mediated oxidative burst
Reactive oxygen species (ROS)	A family of chemicals that are oxidized versions of molecular oxygen, including hydrogen peroxide, superoxide and hydroxyl radicals. ROS are produced by neutrophils via the action of NADPH oxidase and exert antimicrobial activities by damaging lipids, carbohydrates, proteins and nucleic acids

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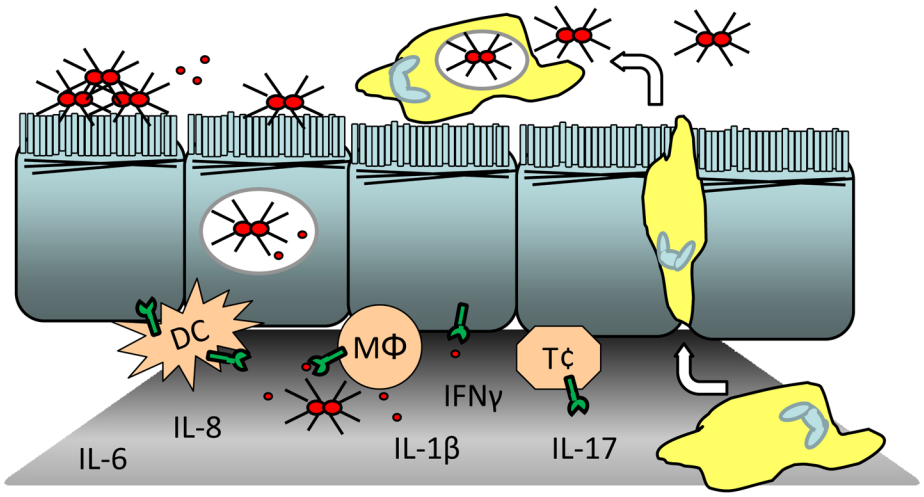

Figure 1. Neutrophil recruitment to sites of infection by the pathogenic neisseriae

Although pathogenic neisseriae colonize diverse mucosal surfaces in the human body, we have only a limited understanding of how differences in these diverse environments create challenges and opportunities for the bacteria [4, 7]. Pathogenic neisseriae use type IV pili, opacity-associated (Opa) proteins and other adhesins to colonize and occasionally invade the mucosal epithelium. Bacterial products such as lipo-oligosaccharide (LOS), peptidoglycan and lipoproteins, which are released either as free compounds or in outer-membrane vesicles (small red circles), stimulate NOD-like receptor (NLR) and Toll-like receptor (TLR) family pattern recognition receptors (green) on epithelial cells and immune cells such as dendritic cells (DCs), macrophages (Mϕ) and T cells (T¢). As a result, gradients of pro-inflammatory cytokines, including interleukin-6 (IL-6), IL-8, IL-1β, interferon-γ (IFNγ) and IL-17, are established. The cytokines recruit neutrophils (yellow) and induce their migration across the epithelium, where they bind and phagocytose the bacteria.

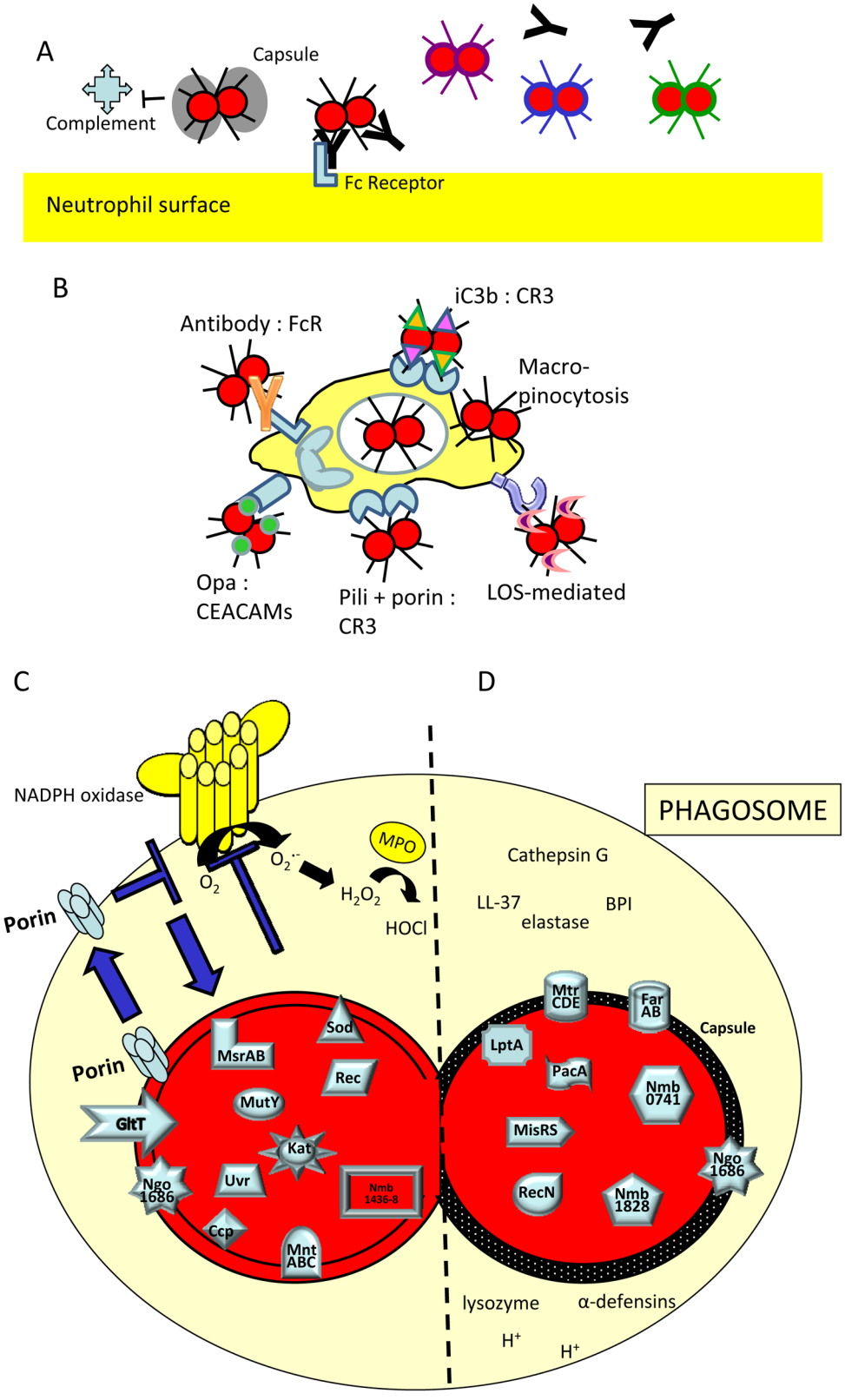

Figure 2. Interactions of pathogenic neisseriae with neutrophils

A. Avoidance of phagocytosis. Capsular polysaccharides (bacterium with black hatched or grey surface) prevent deposition of complement on *Neisseria meningitidis* and subsequent phagocytosis by neutrophils. Continual changes in surface antigens (bacteria with different color surfaces) allow neisseriae to avoid triggering the generation of effective opsonic antibodies that would facilitate phagocytosis.

B. Induction of phagocytosis. Neisseriae can be phagocytosed by host cells using six different routes, which can be receptor dependent or receptor independent: immunoglobulin G (IgG)-opsonized bacteria bind the Fcγ receptor (FcR); proteolytically inactive C3b (iC3b)-opsonized bacteria bind complement receptor 3 (CR3); pili and porin proteins cooperatively bind CR3; neisserial opacity-associated (Opa) proteins bind CEACAMs; lipo-oligosaccharide (LOS) binds unknown receptors on neutrophils; and receptor-independent macropinocytosis can also occur.

C. Protection against neutrophil-mediated antibacterial activities. Neutrophils produce reactive oxygen species (ROS) through the phagocyte NADPH oxidase and myeloperoxidase (MPO). Neisserial proteins such as catalase (KatA), superoxide dismutase (Sod) proteins, the Mnii transport system (MntABC) and l-glutamate transporter (GltT) detoxify or quench ROS. The bacteria also repair proteins damaged by ROS through methionine sulphoxide reductase (MsrAB), and repair DNA damaged by ROS through recombinational (RecA), nucleotide excision (the Uvr proteins) and base excision (MutY) repair of DNA. The pathogenic neisseriae also suppress neutrophil-mediated production of ROS through porins and other mechanisms that are dependent on live bacteria.

D. Bacterial defences against non-oxidative factors from neutrophils. Antimicrobial factors that are independent of ROS include antimicrobial peptides (AMPs), proteases, lysozyme and acid. The multiple transferable resistance system (MtrCDE) and fatty acid resistance system (FarAB) in pathogenic neisseriae remove some of these products from the bacterial cytosol. Furthermore, bacterial lipopolysaccharide transporter periplasmic protein A (LptA) and peptidoglycan *O*-acyltransferase (PacA) modify LOS and peptidoglycan (PG), respectively, to protect the bacteria from these factors. The bacterial proteins NGO1686, RecN, NMB0741 and NMB1828 also protect the bacteria against non-oxidative factors, but their protective mechanisms are unknown. Specific locations of molecules associated with the bacterial envelope (PG, LptA, LOS, NGO1686, MtrCDE, FarAB and MisS) are not shown. HOCl, hypochlorous acid.

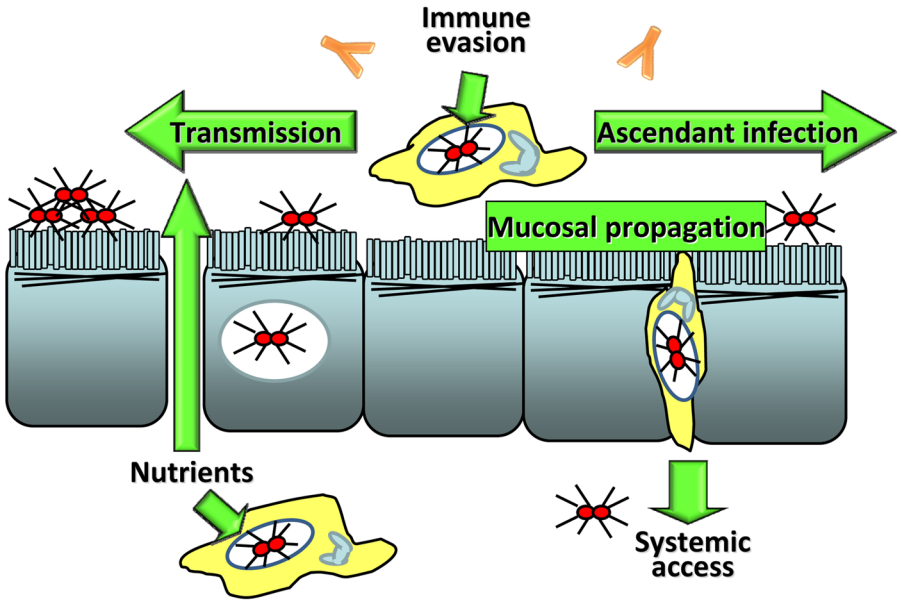

Figure 3. Model for the role of neutrophils in the dissemination and spread of pathogenic neisseriae

Perturbations in the epithelium during neutrophil infiltration enhance the influx of serum and associated nutrients for extracellular neisseriae. Neisseriae that have been phagocytosed by neutrophils may be able to access nutrients from inside the phagosome. Intracellular neisseriae avoid surveillance by humoral immunity. Attachment or phagocytosis by motile neutrophils promotes bacterial movement to deeper or ascendant tissues and transmission to new hosts.

Table 1

Virulence factors of the pathogenic *Neisseria.*

Virulence factor	Presence in *Neisseria*	Function	References
Capsule	*N. meningitidis*	Protects against phagocytosis, antimicrobial peptides, killing by complement. Different strains produce different capsules.	40–42, 47, 59
KatA	Pathogenic and commensal *Neisseria*	Detoxifies H2O2. Protects against ROS. Aids infection of the mouse genital tract.	109, 114, 125, 128
FarAB	Pathogenic and commensal *Neisseria*	Efflux pump that protects bacteria from fatty acids.	133
FHBP	*N. meningitidis* and some commensals	Binds complement factor H to prevent complement-mediated killing.	54
HpuA, HpuB and HmbR	Pathogenic and commensal *Neisseria*	Receptors for haptoglobin-hemoglobin; iron acquisition.	142
IgA protease	Pathogenic *Neisseria* (and one *N. lactamica* isolate)	Cleaves secretory IgA. Cleaves lysosomal LAMP1 in epithelial cells.	154–157
LOS	Pathogenic and commensal *Neisseria*	Endotoxin. Highly stimulatory to the human immune system. Protects against antimicrobial peptides. Adheres to the asialoglycoprotein receptor on urethral cells. Important for phagocytosis by neutrophils. LOS sialylation (by the enzyme Lst) prevents complement deposition and phagocytosis by neutrophils. LOS modification by phosphoethanolamine (by the enzyme LptA) provides resistance to antimicrobial peptides and complement. Strains of the same species produce different LOS glycoforms.	15–16, 20, 27, 47–51, 53, 59, 61, 91, 136–138
LbpAB	Pathogenic and commensal *Neisseria*	Receptor for human lactoferrin; iron acquisition.	142
MntABC	Pathogenic *Neisseria,* some commensals	Quenches ROS.	111, 123, 125
MtrCDE	Pathogenic and commensal *Neisseria*	Efflux pump that protects bacteria from antibiotics and antimicrobial peptides; aids infection of the mouse genital tract.	132, 134
MsrAB	Pathogenic and commensal *Neisseria*	Repairs oxidized proteins.	112
NadA	*N. meningitidis*	Adhesin and invasin.	19
Ngo1686	Pathogenic *Neisseria;* some commensals	Metalloproteinase that protects bacteria from ROS and non-oxidative killing by neutrophils.	38, 39
NMB0741, NMB1828	Pathogenic and commensal *Neisseria*	Hypothetical proteins that protect bacteria from antimicrobial peptides.	139
NMB1436–1438	*N. meningitidis*	Protects against killing by neutrophils.	119
NspA	*N. meningitidis*	Outer membrane protein that binds complement factor H.	55
Opa proteins	Pathogenic *Neisseria* and some commensals (limited repertoire)	Promote attachment and invasion of human cells, including neutrophils; bacterial aggregation; intrastrain changes in Opa expression occur as a result of gene phase variation.	9, 11–13, 20, 36, 44–45, 59, 62–90, 124
Opc	*N. meningitidis*	Structurally related to Opa proteins. Binds vitronectin. Promotes invasion of human cells via integrins.	18
PacA	Pathogenic *Neisseria;* some commensals	Acetylates peptidoglycan to protect from degradation by lysozyme.	140
Type IV pili	Pathogenic *Neisseria* and some commensals	Mediate attachment to various cells/tissues; microcolony formation; twitching motility; natural competence. Extensive intrastrain phase and antigenic variation occurs.	9–10, 17, 35, 43–45, 59, 152–153

Virulence factor	Presence in *Neisseria*	Function	References
Porins	Pathogenic and commensal *Neisseria*	Nutrient acquisition. Recognized by TLR2. Bindscomplement factors C3b, C4b and H, and complement factor 4b-binding protein. Cooperate with pili for CR3- mediated internalization. Can translocate into host cells and modulate ROS production and apoptosis. Strains of the same species can express different porins.	17, 25, 52, 56–57, 61, 100–102, 104, 122
RecN	Pathogenic and commensal *Neisseria*	Recombinational repair protein that protects against ROS and non-oxidative killing by neutrophils.	38–39
SOD proteins	Pathogenic and commensal *Neisseria*	Detoxify ROS.	110, 113, 123
TbpAB (also known as Tbp1–Tbp2)	Pathogenic and commensal *Neisseria*	Receptor for human transferrin; iron acquisition.	142

CR3, complement receptor 3; FarAB, fatty acid resistance system; Fhbp, factor H-binding protein; HmbR, haemoglobin receptor; Hpu, haemoglobin–heptaglobin utilization; IgA, immunoglobulin A; IgAP, IgA1 protease; KatA, catalase; LAMP1, lysosome-associated membrane glycoprotein 1; LbpAB, lactoferrin-binding protein complex; LOS, lipo-oligosaccharide; MntABC, Mn(II) transport system; MsrAB, methionine sulphoxide reductase; MtrCDE, multiple transferable resistance system; NadA, neisserial adhesin A; NspA, neisserial surface protein A; Opa, opacity-associated; Opc, outer-membrane protein C; PacA, peptidoglycan *O*-acyltransferase; ROS, reactive oxygen species; Sod, superoxide dismutase; TbpAB, transferrin-binding protein complex; TLR2, Toll-like receptor 2.