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Gonococcal defenses against antimicrobial activities of neutrophils

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Abstract

Neisseria gonorrhoeae initiates a strong local immune response that is characterized by copious recruitment of neutrophils to the site of infection. Neutrophils neutralize microbes by mechanisms including phagocytosis, extracellular trap formation, production of reactive oxygen species, and the delivery of antimicrobial granular contents. However, neutrophils do not clear infection with *N. gonorrhoeae*. *N. gonorrhoeae* not only expresses factors that defend against neutrophil bactericidal components, but it also manipulates neutrophil production and release of these components. In this review, we will highlight the numerous approaches used by *N. gonorrhoeae* to survive exposure to neutrophils both intracellularly and extracellularly. These approaches reflect the exquisite adaptation of *N. gonorrhoeae* to its obligate human host.

Keywords

neutrophil; *Neisseria gonorrhoeae*; antimicrobial; phagocytosis

NEISSERIA GONORRHOEAE: A MASTER MANIPULATOR OF THE IMMUNE RESPONSE TO INFECTION

Neisseria gonorrhoeae, or the gonococcus (Gc), is a Gram-negative diplococcus that is the causative agent of clinical gonorrhea. Gonorrhea is transmitted via sexual contact with an infected individual, and there is no reservoir for Gc outside the human body. If the infection is left untreated, severe complications such as pelvic inflammatory disease and infertility can occur. Over the past several decades, Gc has acquired resistance to essentially every antibiotic that has been introduced to combat it, leading to the designation of Gc as one of the top three “urgent threats” on the United States Centers for Disease Control and Prevention’s list of antimicrobial resistant pathogens [1].

The control of gonorrhea is complicated by the lack of protective immunity against reinfection, a problem also impeding the development of an effective vaccine and the

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concurrent ability of Gc to evade immunological clearance. Indeed, Gc has a remarkable ability to direct an immune response beneficial for the bacterium while also employing various defenses against immune-mediated killing. For instance, to avoid antibody **opsonization** (see glossary), Gc undergoes high-frequency variation of antigenic surface structures important for infection. This includes **antigenic variation** of type IV pili and **lipooligosaccharide (LOS)**, allelic diversity of the major outer membrane PorB **porin**, and **phase variation** of **opacity-associated (Opa) proteins** (reviewed in [2–4]). Furthermore, antibodies raised against the reduction-modifiable protein (Rmp) on the Gc surface block the antibacterial activity of other anti-gonococcal antibodies, although the underlying mechanism remains enigmatic [5]. Moreover, Gc stimulates high levels of interleukin-10 that, along with regulatory **T cells**, suppresses T-cell-mediated immunity [6]. Instead, Gc induces a Th-17-like immune response through **transforming growth factor-beta (TGF- β)** regulated mechanisms, leading to the abundant recruitment of neutrophils to the urethra, cervix, pharynx, rectum, and conjunctiva [7]. Th17 cells at mucosal sites respond to Gc LOS by producing IL-17A, which initiates the production of cytokines and chemokines that enable neutrophil egress from the bone marrow and migration to infected tissues [7–10]. We recently reported that infected human endocervical cells release the neutrophil chemokine interleukin-8 as well as eicosanoids that drive neutrophil transepithelial migration [11].

Neutrophils are the most abundant leukocyte in human blood and contain a variety of mechanisms to kill microorganisms. Gonorrheal exudates contain significant amounts of neutrophils, many of which are associated with bound and intracellular Gc (Figure 1), and Gc can be readily cultured from neutrophil-rich exudates [12]. Pioneering work from Harry Smith and colleagues showed that Gc is phagocytosed by human neutrophils *ex vivo* and *in vivo* and survives inside them [13–15]. These observations imply that the neutrophilic immune response to Gc is not effective, despite neutrophils having multiple mechanisms for bacterial killing. These mechanisms include phagocytosis of bacteria, fusion of antimicrobial containing granules to the bacterial phagosome, production of **reactive oxygen species (ROS)**, and release of neutrophil extracellular traps. As we will discuss below, Gc uses multiple approaches to enhance its survival in the presence of neutrophils, facilitating bacterial persistence and spread within and between hosts. In this review, we will highlight recent findings that how the bacteria evade neutrophil-mediated killing (Figure 2, key figure).

EVASION AND MANIPULATION OF NEUTROPHIL PHAGOCYTOSIS

Although intracellular gonococci are observed within neutrophils from gonorrheal exudates, Gc can limit and redirect neutrophil opsonic and non-opsonic phagocytosis (Figure 3). As described above, phase and antigenic variation along with expression of Rmp limit antibody-mediated recognition of Gc and the potential for Fc receptor-mediated opsonophagocytosis [5]. However, we reported that Gc opsonized with a rabbit polyclonal antibody was avidly phagocytosed and killed by adherent, primary human neutrophils [16]. This finding implies that if a vaccine against Gc that stimulates an Rmp-independent antibody response can be developed, opsonophagocytosis by neutrophils and other phagocytes may be critical to its efficacy. In support of this possibility, neutrophils contributed to killing of Gc *in vitro* when

the bacteria were opsonized with an experimental vaccine candidate directed against the β -chain lactose of Gc LOS [17].

Gc also employs mechanisms to limit **complement** deposition on its surface, thus evading complement-mediated opsonophagocytosis. In particular, porin B, the most abundantly expressed outer membrane protein found on Gc, binds to C4 binding protein and factor H, which limit the classical and alternative pathways of complement activation, respectively (Figure 3) [18]. Sialylation of the lacto-*N*-tetraose moiety on the α chain of LOS enhances factor H deposition on porin, explaining in part the increased resistance of sialylated Gc to phagocytosis [19].

Phase variation of Gc surface structures influences non-opsonic interactions with neutrophils. In particular, non-opsonic phagocytosis of Gc by human neutrophils is mediated by Opa proteins that interact with carcinoembryonic antigen related cellular adhesion molecules (CEACAMs) [20]. Gc strains possess ~11–13 *opa* genes, each of which is phase-variable. Most Opa proteins interact with one or more CEACAMs, which serve as adhesins and invasins for neutrophils and epithelial cells. The features of Opa proteins that dictate receptor specificity are independent of primary sequence and instead result from conformations achieved by their extracellular loops [21]. Among the CEACAMs expressed on human neutrophils, CEACAM-3 contains an immunotyrosine activation motif and initiates pro-inflammatory signaling cascades in conjunction with **Toll-Like Receptor (TLR)** signaling through Bcl10 [22–24]. CEACAM-3 is unique to granulocytes, implying that it has evolved to promote bacterial killing. We and others have shown that Opa+ Gc that engage CEACAM-3 are readily phagocytosed, induce a potent oxidative burst, and are killed by human neutrophils [16, 25, 26]. Epidemiological evidence suggests that the Gc *opa* genes are under evolutionary selective pressure to avoid binding to CEACAM-3 while maintaining binding to CEACAM-1 and other CEACAMs that assist in epithelial colonization [27, 28].

Whether opsonic or non-opsonic, Gc avoidance of phagocytosis may be enhanced by phase-variable expression of type IV pili, which facilitate Gc attachment to the uropod of migrating neutrophils to escape phagocytosis at the cells' leading edge [29]. Together, these findings indicate a subset of Gc in a population has reduced phagocytic interactions with human neutrophils, implying those bacteria have a relative survival advantage.

Notably, the extent of Gc phagocytosis is influenced by the physiological state of the neutrophils. Early studies used Gc and neutrophils maintained in suspension in buffered saline, in which neutrophils are poorly activated. Under these conditions, Gc required opsonization and/or Opa expression in order to associate with neutrophils (reviewed in [30]). In contrast, neutrophils can phagocytose Opa-negative, unopsonized Gc when the cells are adherent and incubated with chemokines to mimic their behavior after migration into tissues [31, 32]. Future studies should investigate phagocytosis and other functions of neutrophils in more nuanced model systems, including after migration across human epithelial monolayers [11] and into mouse tissues [33, 34].

MANIPULATION OF PHAGOSOME MATURATION

Downstream of phagocytosis, Gc limits neutrophil phagosome maturation in order to enable bacterial intracellular survival. In neutrophils, the nascent phagosome matures by fusion with cytoplasmic granules [35]. Neutrophils make different subsets of granules, each with distinct antimicrobial cargo (Table 1). Of these subsets, primary granules contain the most anti-gonococcal agents, which are released last. Opa- Gc and Gc that are serum (complement)-opsonized are internalized into phagosomes that lack primary granule components (i.e., immature phagosome) [31]. Thus the bacteria are not exposed to these degradative components early in infection. Residence within an immature phagosome correlates with enhanced bacterial viability, and treating neutrophils with lysophosphatidylcholine to promote primary granule fusion with the Gc phagosome decreases viability of Opa- bacteria [16]. Although the Gc phagosome eventually fuses with primary granules, live bacteria are recovered from neutrophils at these later times. Gc may exploit a delay in phagosome maturation in order to upregulate its antimicrobial defenses as an adaptation to the potentially toxic environment inside neutrophils, or more generally in its human host.

Importantly, Opa- bacteria that are heat-killed prior to exposure to neutrophils also reside in immature phagosomes, indicating that manipulation of phagosome maturation does not rely on active Gc processes [25]. This is in keeping with the observation that Gc does not encode secretion systems that inject proteins to manipulate host cell functions [36, 37]. Instead, this finding suggests that components on the surface of Gc that interface with neutrophil phagocytic receptors may influence phagosome maturation. In support of this suggestion, Opa+ Gc that engage CEACAM-3 or IgG-opsonized Gc that engage Fc receptors are internalized by neutrophils into mature phagolysosomes, where bacterial killing is enhanced (Figure 4). The antigenococcal activity of primary granules is attributed in part to serine proteases that degrade bacterial membrane proteins as well as process antimicrobial peptides into their mature, active form. Src and Syk kinase activities are implicated in primary granule mobilization to neutrophil phagosomes [33, 38, 39].

Changes to the bacterial cell envelope influence the maturity of the Gc phagosome and thus bacterial intracellular survival. As one example, Gc modifies LOS by addition of phosphoethanolamine (PEA) to the 4' phosphate on lipid A, catalyzed by the phase-variable LOS phosphoethanolamine transferase A (LptA) [40, 41]. PEA decoration enhances interactions of lipid A with TLR4, increasing pro-inflammatory cytokine production and cervicovaginal influx of neutrophils in mice following Gc infection [42, 43]. Furthermore, modification of LOS by LptA decreases the negative envelope charge of Gc and thus enhances the resistance of Gc to killing by cationic antimicrobial peptides (CAMPs) [44, 45]. Gc lacking *lptA* is attenuated *in vivo* in a human male urethral-infection model and in a mouse model of Gc cervicovaginal colonization [46]. Moreover, *lptA* mutant Gc is more likely to reside in primary granule-positive phagolysosomes, an observation linked with a significant reduction in bacterial survival compared with parent Gc [40]. As another example, Gc has a unique peptidoglycan (PG) turnover machinery, in that PG monomers are released extracellularly rather than efficiently recycled [47]. Monomer generation is catalyzed by two lytic transglycosylases, LtgA and LtgD [48] (see 'Peptidoglycan

dynamics'). Deletion of these transglycosylases rendered Gc more sensitive to killing by neutrophils and more likely to traffic to more mature phagosomes. Because both LOS and PG are recognized by TLR and **Nod-Like Receptor (NLR)** innate immune receptors expressed by neutrophils, signaling through those receptors may more generally modulate granule mobilization to phagosomes, but this possibility remains to be fully explored.

There are three major open questions about the connection between phagocytosis and phagosome maturation that are relevant for Gc infection. First, how do phagocytosed Opa- and serum-opsonized bacteria avoid delivery into phagolysosomes? The observation that unopsonized, Opa- Gc is internalized by neutrophils runs counter to dogma in the field that phagocytosis of Gc requires opsonization or expression of Opa proteins [49, 50]. This discrepancy may be related to the use of adherent, chemokine-primed neutrophils to demonstrate Opa- Gc phagocytosis, as mentioned above. Second, what is the phagocytic fate of Opa+ Gc that do not bind to CEACAM-3? CEACAM-1 has an immunotyrosine inhibitory motif and might be expected to inhibit phagosome maturation [51]. However, Sarantis and Gray-Owen reported that bacteria expressing a CEACAM-1 binding Opa protein transactivated CEACAM-3 in mouse promyelocytes, one caveat being that these human receptors were overexpressed following transfection [52]. Third, how does manipulating phagosome maturation affect Gc survival within neutrophils long-term? Neutrophils are short-lived, terminally differentiated cells. Although Gc delays the spontaneous apoptosis of neutrophils [53, 54], the bacteria must eventually exit or will die within them. Answering these questions will give important insight into the contributions of Opa proteins to Gc pathogenesis, in particular the neutrophilic inflammatory response.

EVASION OF NEUTROPHIL EXTRACELLULAR TRAPS

First described in a seminal paper by the Zychlinsky group in 2004, neutrophil extracellular traps (NETs) are structures composed of DNA that is covered in histones and other CAMPs that are released from neutrophils [55]. NETs have been shown to capture and kill a variety of microbes, including pathogenic bacteria, but are also implicated in autoimmune disorders due to the display of potential self-antigens [55, 56]. Canonical NET release via phorbol esters requires ROS production and a cell death pathway termed NETosis. However, NET release without cell death has also been reported [57]. Infection with Gc induces NET release from human neutrophils under conditions where the oxidative burst does not occur, suggesting an alternative pathway of NET formation that remains to be elucidated [58].

Gc survives in the presence of NETs, whether the NETs are induced by Gc or ectopically stimulated. We found that Gc releases Nuc, a thermonuclease that degrades the NET DNA backbone (Figure 5) [59]. Gc in which the *nuc* gene was deleted were more susceptible to killing by NETs compared with wild-type Gc. Survival of Nuc deficient Gc was restored upon addition of DNase, purified Nuc, and bacterial supernatants from Nuc-expressing bacteria [59]. Nuc is present in all Gc strains examined to date, suggesting conserved roles in Gc biology; in fact, Nuc is also important for remodeling of the DNA-based scaffold of Gc biofilms to facilitate efficient bacterial colonization [60, 61].

NETs kill microbes in two ways: by increasing the local concentration of CAMPs and by sequestering essential metals through proteins like calprotectin (S100A8/A9) [62]. Gc has evolved ways to overcome both these antimicrobial strategies. LptA (see 'Manipulation of Phagosome Maturation') is also critical for survival of Gc in the presence of NETs, likely due to susceptibility of *lptA* mutant Gc to CAMPs contained within NETs [63]. More recently, we found that the surface-exposed TonB-dependent transporter TdfH enhances Gc survival in NETs by overcoming host sequestration of the essential trace metal zinc [63]. NETs are decorated with calprotectin, which binds zinc and manganese in a calcium-dependent manner. TdfH binds to human calprotectin and extracts zinc from it [63], analogous to interactions of Gc TbpA with human transferrin for iron acquisition [64]. *tdfH* mutant Gc was more susceptible to NET-mediated killing compared to wild-type Gc, and resistance of *tdfH*-deficient Gc to NETs was restored by addition of exogenous zinc. Other defenses against neutrophil antimicrobial proteins, described below, may also have roles in protecting Gc from killing in NETs.

SUPPRESSION OF REACTIVE OXYGEN SPECIES PRODUCTION

In neutrophils, signaling in response to extracellular stimuli leads to the co-assembly of the cytoplasmic and membrane-associated subunits of the **NADPH oxidase** enzyme [65]. Once fully formed, NADPH oxidase converts molecular oxygen to superoxide anion, which dismutates into hydrogen peroxide. The primary granule enzyme myeloperoxidase can then convert hydrogen peroxide into hypochlorous acid, an extremely potent oxidant. As described above, Gc manipulates delivery of primary granule contents into its phagosome, to limit myeloperoxidase release. Engagement of CEACAM-3 by Opa proteins stimulates a potent oxidative burst in neutrophils, which is directed particularly into the phagosome containing Gc [25, 33]. In contrast, neutrophils exposed to Opa- Gc do not generate ROS [32]. Specifically, the cytosolic NADPH oxidase subunits p40, p47, and p67 were not recruited to phagosomal or plasma membranes, where the membrane-associated subunits gp91 and p22 were found [25]. This was correlated with an absence of p47 phosphorylation, a prerequisite to assembly of the holoenzyme. Moreover, Opa- Gc inhibits the oxidative burst in human neutrophils exposed to stimuli such as formylated peptides and *Staphylococcus aureus* [16, 32]. The mechanism behind the suppression remains to be elucidated, but it requires live, metabolically active Gc. *Neisserial* porin has been reported to translocate into the mitochondria of neutrophils and thereby inhibit the oxidative burst [66]. Because porin is essential in Gc, these studies used purified porin that was added to human cells. In contrast, PorB of the related pathogen *Neisseria meningitidis* had no role in inhibiting the neutrophil oxidative burst, and mutagenesis of Gc PorB did not reveal any protein domains that were necessary for blocking neutrophil ROS production [67, 68]. Thus, how Gc manipulates neutrophil oxidative functions remain enigmatic. Moreover, as we will discuss below, the role of ROS in the anti-gonococcal activity of neutrophils is questionable.

DEFENSES AGAINST NEUTROPHIL ANTIMICROBIAL CONTENTS

During infection, Gc is exposed to the antimicrobial components of neutrophils in phagosomes and in the extracellular space, free or associated with NETs. Neutrophil primary, secondary, and tertiary granules contain unique sets of antimicrobial contents

(Table 1) [69, 70]. These components fall into two categories: oxidative species and non-oxidative killing proteins and peptides. Given their long co-evolution with neutrophils, it is not surprising that Gc has evolved numerous ways to defend itself against neutrophil antimicrobial components. However, it is important to note that not all bacterial factors that assist in defense from antimicrobial components protect from neutrophil-mediated killing, as these components may also be found in mucosal cells and secretions that Gc encounters.

Antioxidant defenses

As an aerobic bacterium, Gc expresses numerous defenses against ROS, including catalase, superoxide dismutase, glutathione peroxidases, a manganese import system, oxidative DNA damage repair enzymes, and methionine sulfoxide reductase [71–74]. However, none of these defenses, singly or together, contribute to Gc survival in the presence of neutrophils, even when a strong oxidative burst is generated [25, 75, 76]. These observations are in line with seminal reports from the Rest and Shafer groups indicating that ROS is dispensable for neutrophil antigenococcal activity [77, 78].

Gc upregulates numerous genes following exposure to hydrogen peroxide [79]. Two of these genes, *ngo1686* and *recN*, confer resistance to both ROS and killing by neutrophils. *ngo1686* encodes a zinc metalloprotease, later named Mpg, which processes the peptide stems of PG [80]. Mpg expression facilitates full surface presentation of the type IV pilus, a major bacterial adhesin, which was then found to defend Gc from hydrogen peroxide and killing by neutrophils [80]. However, the sensitivity of the *ngo1686* mutant to neutrophils was not restored using neutrophils that cannot make ROS. In fact, Gc mutants in *ngo1686* and the type IV pilus were also more sensitive to LL-37 (see ‘Defenses against cationic antimicrobial peptides’). *recN* also conferred resistance to hydrogen peroxide and neutrophils, but susceptibility of a *recN* mutant to neutrophils was ROS-independent. These findings suggest that Gc senses oxidative stress and responds by upregulating gene products that can defend against a variety of host immune components, including neutrophils themselves. However, they also emphasize that “correlation is not causation,” and mechanisms of resistance to individual antimicrobial components may not necessarily be phenocopied in the context of infection.

Defenses against cationic antimicrobial peptides

During infection, Gc is exposed to two major families of cationic antimicrobial peptides, the defensins and the cathelicidin LL-37. These peptides are made by neutrophils and also found in mucosal secretions at sites Gc infects in the human body. A peptide derived from the serine protease cathepsin G also has antimicrobial activity [81, 82]. As described above, the LOS-modifying enzyme LptA and the PG-modifying enzyme Mpg help confer resistance to CAMPs and to neutrophils [40, 80]. The MtrCDE efflux pump exports CAMPs as well as antibiotics and toxic dyes from the Gc cytoplasm and is crucial for Gc survival in the mouse genital tract [83]. Gc detects increasing levels of CAMPs using the MisRS two-component system. The MisR response regulator controls expression of 94 target genes, 59% of which are involved in envelope integrity of Gc, implying that the MisR response to high levels of CAMPs is to decrease the ability of these antimicrobial peptides to enter Gc [84].

Nutrient Acquisition Factors

One of the most striking features of Gc is its ability to scavenge essential metals from the host proteins that are meant to sequester them from invading microorganisms [85]. This activity is predominantly carried out by transporters that use the energy of the TonB-ExbB-ExbD system to shuttle metals across the outer membrane and transfer them to inner membrane transport systems for delivery to the cytoplasm [86]. As described above, Gc uses the TonB-dependent transporter TdfH to extract zinc from the abundant neutrophil protein calprotectin in order to survive in NETs [63]. Interestingly, TdfH is part of the MisR regulon in strain FA19, suggesting a connection between CAMP sensing and nutrient acquisition (Figure 5) [84]. There are additional TonB-dependent transporters in Gc that contribute to metal acquisition, including the zinc transporter TdfJ, and TbpAB and LbpAB, which access iron from human transferrin and lactoferrin, respectively [63, 87]; however, their roles in Gc survival from neutrophils has not yet been reported.

Neutrophils predominantly use glycolysis for ATP production, through which D-lactate is produced [88]. Gc encodes two D-lactate dehydrogenases, one cytoplasmic (LdhA) and the other membrane bound (LdhD), as well as an L-lactate dehydrogenase (LldD) [89]. LdhA and LdhD contribute to the prolonged survival of Gc inside neutrophils. The female genital tract also has high levels of lactate produced by the resident microbiota, suggesting lactate utilization affords Gc an additional metabolic niche *in vivo* [90]. Interestingly, exposure to lactate stimulates Gc oxidative metabolism and has been proposed as a mechanism to thwart neutrophil ROS production [91, 92]. Thus utilization of lactate can enhance Gc survival during infection in multiple, non-redundant ways.

Peptidoglycan dynamics

PG turnover in Gc is unique from other Gram-negative bacteria in that PG fragments are poorly recycled from the periplasm and instead released extracellularly [47]. As described above, one of the first Gc virulence factors shown to protect against killing by neutrophils was NGO1686/Mpg, a PG peptidase, involved in PG turnover (Figure 5) [80]. More recently, Gc lytic transglycosylases LtgA and LtgD, which are responsible for release of immunomodulatory PG monomer fragments, confer protection against killing by neutrophils, and specifically against lysozyme and the serine protease neutrophil elastase [48]. The mechanism of protection is likely an increase in the amount of envelope integrity of Gc. High concentrations of lysozyme are found in phagocytes like neutrophils and also mucosal secretions [93]. As for other microbes, lysozyme resistance in Gc is multi-factorial [93]. Additional lysozyme resistance factors that contribute to Gc survival from neutrophils include NGO1063/SliC and NGO1981, proteinaceous lysozyme inhibitors [94–96], and PacA and PacB, which O-acetylate peptidoglycan [48, 97].

Other bacterial proteins

Macrophage infectivity potentiator (MIP) proteins are made by multiple species of bacteria. They are surface-exposed, have peptidyl-prolyl isomerase activity, and aid in evasion of killing by macrophages [98]. Reimer *et al.* recently reported that the Gc MIP homolog also aids in defense from killing in neutrophil phagosomes [99]. How the biochemical activity of MIP relates to resistance to phagocytic killing remains to be elucidated.

CONCLUDING REMARKS

The obligate human pathogen Gc has evolved a remarkable lifestyle in which it stimulates the recruitment of neutrophils and survives in their midst. The approaches used by Gc to survive from neutrophils combine a sophisticated offense (manipulating neutrophil functionality) with a strong defense (expressing defenses against neutrophil antimicrobial components) (Figure 2). Several outstanding questions about these survival strategies remain to be elucidated (see Outstanding Questions). Features complicating these studies include the exquisite adaptation of Gc to its obligate human host and the fact that primary neutrophils have a short lifespan and cannot be genetically manipulated in their terminally differentiated form in the blood. New experimental approaches including *ex vivo* generation of neutrophils from pluripotent stem cells and mouse strains that are transgenic for human factors that promote Gc infection will help to address these gaps [52, 100]. Understanding the mechanisms by which Gc resists killing by neutrophils can reveal targets for novel antimicrobials that render Gc susceptible to these abundant antimicrobial phagocytes, an attractive therapeutic option to combat the worldwide public health emergency of multidrug-resistant gonorrhea.

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References

1. United States Centers for Disease Control and Prevention. , editorAntibiotic Resistance Threats in the United States. 2013. 1–114. www.cdc.gov/drugresistance/threat-report-2013
2. Criss AK, et al. The frequency and rate of pilin antigenic variation in *Neisseria gonorrhoeae*. *Mol Microbiol.* 2005; 58(2):510–9. [PubMed: 16194236]
3. Murphy GL, et al. Phase variation of gonococcal protein II: regulation of gene expression by slipped-strand mispairing of a repetitive DNA sequence. *Cell.* 1989; 56(4):539–47. [PubMed: 2492905]
4. Schneider H, et al. Instability of expression of lipooligosaccharides and their epitopes in *Neisseria gonorrhoeae*. *Infect Immun.* 1988; 56(4):942–6. [PubMed: 3126149]
5. Plummer FA, et al. Antibody to Rmp (outer membrane protein 3) increases susceptibility to gonococcal infection. *J Clin Invest.* 1993; 91(1):339–43. [PubMed: 8423230]
6. Liu Y, et al. Suppression of host adaptive immune responses by *Neisseria gonorrhoeae*: role of interleukin 10 and type 1 regulatory T cells. *Mucosal Immunol.* 2014; 7(1):165–76. [PubMed: 23757303]
7. Liu Y, et al. *Neisseria gonorrhoeae* selectively suppresses the development of Th1 and Th2 cells, and enhances Th17 cell responses, through TGF-beta-dependent mechanisms. *Mucosal Immunol.* 2012; 5(3):320–31. [PubMed: 22354319]
8. Packiam M, et al. Protective role of Toll-like receptor 4 in experimental gonococcal infection of female mice. *Mucosal Immunol.* 2012; 5(1):19–29. [PubMed: 21937985]
9. Ramsey KH, et al. Inflammatory cytokines produced in response to experimental human gonorrhea. *J Infect Dis.* 1995; 172(1):186–91. [PubMed: 7797909]
10. Feinen B, et al. Critical role of Th17 responses in a murine model of *Neisseria gonorrhoeae* genital infection. *Mucosal Immunol.* 2010; 3(3):312–21. [PubMed: 20107432]

11. Stevens JS, et al. Endocervical and neutrophil lipoxygenases coordinate neutrophil transepithelial migration to *Neisseria gonorrhoeae*. *J Infect Dis*. 2018; doi: 10.1093/infdis/jiy347
12. Johnson MB, Criss AK. Resistance of *Neisseria gonorrhoeae* to neutrophils. *Front Microbiol*. 2011; 2:77. [PubMed: 21747795]
13. Witt K, et al. Resistance of *Neisseria gonorrhoeae* grown in vivo to ingestion and digestion by phagocytes of human blood. *J Gen Microbiol*. 1976; 96(2):341–50. [PubMed: 825610]
14. Casey SG, et al. Demonstration of intracellular growth of gonococci in human phagocytes using spectinomycin to kill extracellular organisms. *J Gen Microbiol*. 1979; 113(2):395–8. [PubMed: 159942]
15. Veale DR, et al. The intracellular survival and growth of gonococci in human phagocytes. *J Gen Microbiol*. 1979; 113(2):383–93. [PubMed: 117078]
16. Johnson MB, Criss AK. *Neisseria gonorrhoeae* phagosomes delay fusion with primary granules to enhance bacterial survival inside human neutrophils. *Cell Microbiol*. 2013; 15(8):1323–40. [PubMed: 23374609]
17. Chakraborti S, et al. Phase-Variable Heptose I Glycan Extensions Modulate Efficacy of 2C7 Vaccine Antibody Directed against *Neisseria gonorrhoeae* Lipooligosaccharide. *J Immunol*. 2016; 196(11):4576–86. [PubMed: 27183633]
18. Ngampasutadol J, et al. Human factor H interacts selectively with *Neisseria gonorrhoeae* and results in species-specific complement evasion. *J Immunol*. 2008; 180(5):3426–35. [PubMed: 18292569]
19. Shaughnessy J, et al. A Novel Factor H-Fc Chimeric Immunotherapeutic Molecule against *Neisseria gonorrhoeae*. *J Immunol*. 2016; 196(4):1732–40. [PubMed: 26773149]
20. Gray-Owen SD, et al. CD66 carcinoembryonic antigens mediate interactions between Opa-expressing *Neisseria gonorrhoeae* and human polymorphonuclear phagocytes. *EMBO J*. 1997; 16(12):3435–45. [PubMed: 9218786]
21. Bos MP. Carcinoembryonic Antigen Family Receptor Recognition by Gonococcal Opa Proteins Requires Distinct Combinations of Hypervariable Opa Protein Domains. *Infect Immun*. 2002; 70(4):1715–1723. [PubMed: 11895933]
22. Sintsova A, et al. Bcl10 synergistically links CEACAM3 and TLR-dependent inflammatory signalling. *Cell Microbiol*. 2018; 20(1)doi: 10.1111/cmi.12788
23. McCaw SE, et al. Immunoreceptor tyrosine-based activation motif phosphorylation during engulfment of *Neisseria gonorrhoeae* by the neutrophil-restricted CEACAM3 (CD66d) receptor. *Mol Microbiol*. 2003; 49(3):623–37. [PubMed: 12864848]
24. Schmitter T, et al. Granulocyte CEACAM3 is a phagocytic receptor of the innate immune system that mediates recognition and elimination of human-specific pathogens. *J Exp Med*. 2004; 199(1): 35–46. [PubMed: 14707113]
25. Smirnov A, et al. Assembly of NADPH oxidase in human neutrophils is modulated by the opacity-associated protein expression state of *Neisseria gonorrhoeae*. *Infect Immun*. 2014; 82(3):1036–44. [PubMed: 24343654]
26. Criss AK, Seifert HS. *Neisseria gonorrhoeae* suppresses the oxidative burst of human polymorphonuclear leukocytes. *Cell Microbiol*. 2008; 10(11):2257–70. [PubMed: 18684112]
27. Sintsova A, et al. Selection for a CEACAM receptor-specific binding phenotype during *Neisseria gonorrhoeae* infection of the human genital tract. *Infect Immun*. 2015; 83(4):1372–83. [PubMed: 25605771]
28. Islam E, et al. Specific binding to differentially-expressed human CEACAMs determines the outcome of *Neisseria gonorrhoeae* infections along the female reproductive tract. *Infect Immun*. 2018; doi: 10.1128/IAI.00092-18
29. Soderholm N, et al. Pathogenic *Neisseria* Hitchhike on the Uropod of Human Neutrophils. *PLoS One*. 2011; 6(9):e24353. [PubMed: 21949708]
30. Criss AK, Seifert HS. A bacterial siren song: intimate interactions between *Neisseria* and neutrophils. *Nat Rev Microbiol*. 2012; 10(3):178–90. [PubMed: 22290508]
31. Criss AK, et al. Resistance of *Neisseria gonorrhoeae* to non-oxidative killing by adherent human polymorphonuclear leucocytes. *Cell Microbiol*. 2009; 11(7):1074–87. [PubMed: 19290914]

32. Ball LM, Criss AK. Constitutively Opa-expressing and Opa-deficient *Neisseria gonorrhoeae* strains differentially stimulate and survive exposure to human neutrophils. *J Bacteriol.* 2013; 195(13): 2982–90. [PubMed: 23625842]
33. Sintsova A, et al. Global analysis of neutrophil responses to *Neisseria gonorrhoeae* reveals a self-propagating inflammatory program. *PLoS Pathog.* 2014; 10(9):e1004341. [PubMed: 25188454]
34. Islam EA, et al. The reproductive cycle is a pathogenic determinant during gonococcal pelvic inflammatory disease in mice. *Mucosal Immunol.* 2016; 9(4):1051–64. [PubMed: 26693700]
35. Borregaard N, et al. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol.* 2007; 28(8):340–5. [PubMed: 17627888]
36. Hamilton HL, et al. *Neisseria gonorrhoeae* secretes chromosomal DNA via a novel type IV secretion system. *Mol Microbiol.* 2005; 55(6):1704–21. [PubMed: 15752195]
37. Dillard JP, Seifert HS. A variable genetic island specific for *Neisseria gonorrhoeae* is involved in providing DNA for natural transformation and is found more often in disseminated infection isolates. *Mol Microbiol.* 2001; 41(1):263–77. [PubMed: 11454218]
38. Johnson MB, et al. Opa+ *Neisseria gonorrhoeae* exhibits reduced survival in human neutrophils via Src family kinase-mediated bacterial trafficking into mature phagolysosomes. *Cell Microbiol.* 2015; 17(5):648–65. [PubMed: 25346239]
39. Hauck CR, et al. CD66-mediated phagocytosis of Opa52 *Neisseria gonorrhoeae* requires a Src-like tyrosine kinase- and Rac1-dependent signalling pathway. *EMBO J.* 1998; 17(2):443–54. [PubMed: 9430636]
40. Handing JW, Criss AK. The lipooligosaccharide-modifying enzyme LptA enhances gonococcal defence against human neutrophils. *Cell Microbiol.* 2015; 17(6):910–21. [PubMed: 25537831]
41. Zughaier SM, et al. Phosphoethanolamine Modification of *Neisseria gonorrhoeae* Lipid A Reduces Autophagy Flux in Macrophages. *PLoS One.* 2015; 10(12):e0144347. [PubMed: 26641098]
42. Packiam M, et al. Phosphoethanolamine decoration of *Neisseria gonorrhoeae* lipid A plays a dual immunostimulatory and protective role during experimental genital tract infection. *Infect Immun.* 2014; 82(6):2170–9. [PubMed: 24686069]
43. John CM, et al. Lack of lipid A pyrophosphorylation and functional lptA reduces inflammation by *Neisseria* commensals. *Infect Immun.* 2012; 80(11):4014–26. [PubMed: 22949553]
44. Kandler JL, et al. Phase-variable expression of *lptA* modulates the resistance of *Neisseria gonorrhoeae* to cationic antimicrobial peptides. *Antimicrob Agents Chemother.* 2014; 58(7):4230–3. [PubMed: 24820072]
45. Lewis LA, et al. Phosphoethanolamine substitution of lipid A and resistance of *Neisseria gonorrhoeae* to cationic antimicrobial peptides and complement-mediated killing by normal human serum. *Infect Immun.* 2009; 77(3):1112–20. [PubMed: 19114544]
46. Hobbs MM, et al. Lipid A's structure mediates *Neisseria gonorrhoeae* fitness during experimental infection of mice and men. *MBio.* 2013; 4(6):e00892–13. [PubMed: 24255126]
47. Rosenthal RS. Release of soluble peptidoglycan from growing gonococci: hexaminidase and amidase activities. *Infect Immun.* 1979; 24(3):869–78. [PubMed: 112060]
48. Ragland SA, et al. Two lytic transglycosylases in *Neisseria gonorrhoeae* impart resistance to killing by lysozyme and human neutrophils. *Cell Microbiol.* 2017; 19(3)doi: 10.1111/cmi.12662
49. Chen T, Gotschlich EC. CGM1a antigen of neutrophils, a receptor of gonococcal opacity proteins. *Proc Natl Acad Sci U S A.* 1996; 93(25):14851–6. [PubMed: 8962144]
50. Rest RF, et al. Interactions of *Neisseria gonorrhoeae* with human neutrophils: effects of serum and gonococcal opacity on phagocyte killing and chemiluminescence. *Infect Immun.* 1982; 36(2):737–44. [PubMed: 6806195]
51. Gray-Owen SD, Blumberg RS. CEACAM1: contact-dependent control of immunity. *Nat Rev Immunol.* 2006; 6(6):433–46. [PubMed: 16724098]
52. Sarantis H, Gray-Owen SD. Defining the roles of human carcinoembryonic antigen-related cellular adhesion molecules during neutrophil responses to *Neisseria gonorrhoeae*. *Infect Immun.* 2012; 80(1):345–58. [PubMed: 22064717]
53. Chen A, Seifert HS. *Neisseria gonorrhoeae*-mediated inhibition of apoptotic signalling in polymorphonuclear leukocytes. *Infect Immun.* 2011; 79(11):4447–4458. [PubMed: 21844239]

54. Simons MP, et al. *Neisseria gonorrhoeae* delays the onset of apoptosis in polymorphonuclear leukocytes. *Cell Microbiol.* 2006; 8(11):1780–90. [PubMed: 16803582]
55. Brinkmann V, et al. Neutrophil extracellular traps kill bacteria. *Science.* 2004; 303(5663):1532–5. [PubMed: 15001782]
56. Lee KH, et al. Neutrophil extracellular traps (NETs) in autoimmune diseases: A comprehensive review. *Autoimmun Rev.* 2017; 16(11):1160–1173. [PubMed: 28899799]
57. Yipp BG, et al. Infection-induced NETosis is a dynamic process involving neutrophil multitasking in vivo. *Nat Med.* 2012; 18(9):1386–93. [PubMed: 22922410]
58. Gunderson CW, Seifert HS. *Neisseria gonorrhoeae* elicits extracellular traps in primary neutrophil culture while suppressing the oxidative burst. *MBio.* 2015; 6(1):e20452–14.
59. Juneau RA, et al. A Thernonuclease of *Neisseria gonorrhoeae* Enhances Bacterial Escape From Killing by Neutrophil Extracellular Traps. *J Infect Dis.* 2015; 212(2):316–24. [PubMed: 25605868]
60. Falsetta ML, et al. The Composition and Metabolic Phenotype of *Neisseria gonorrhoeae* Biofilms. *Front Microbiol.* 2011; 2:75. [PubMed: 21833322]
61. Steichen CT, et al. The *Neisseria gonorrhoeae* biofilm matrix contains DNA, an endogenous nuclease controls its incorporation. *Infect Immun.* 2011; 79(4):1504–11. [PubMed: 21300774]
62. Urban CF, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog.* 2009; 5(10):e1000639. [PubMed: 19876394]
63. Jean S, et al. *Neisseria gonorrhoeae* Evades Calprotectin-Mediated Nutritional Immunity and Survives Neutrophil Extracellular Traps by Production of TdFH. *Infect Immun.* 2016; 84(10):2982–94. [PubMed: 27481245]
64. Noinaj N, et al. The transferrin-iron import system from pathogenic *Neisseria* species. *Mol Microbiol.* 2012; 86(2):246–57. [PubMed: 22957710]
65. Nauseef WM. Assembly of the phagocyte NADPH oxidase. *Histochem Cell Biol.* 2004; 122(4):277–91. [PubMed: 15293055]
66. Muller A, et al. Targeting of the pro-apoptotic VDAC-like porin (PorB) of *Neisseria gonorrhoeae* to mitochondria of infected cells. *EMBO J.* 2000; 19(20):5332–43. [PubMed: 11032801]
67. Peak IR, et al. *Neisseria meningitidis* Lacking the Major Porins PorA and PorB Is Viable and Modulates Apoptosis and the Oxidative Burst of Neutrophils. *J Proteome Res.* 2016; 15(8):2356–65. [PubMed: 26562068]
68. Chen A, Seifert HS. Structure-function studies of the *Neisseria gonorrhoeae* major outer membrane porin. *Infect Immun.* 2013; 81(12):4383–91. [PubMed: 24042111]
69. Le Cabec V, et al. Targeting of proteins to granule subsets is determined by timing and not by sorting: The specific granule protein NGAL is localized to azurophil granules when expressed in HL-60 cells. *Proc Natl Acad Sci U S A.* 1996; 93(13):6454–7. [PubMed: 8692836]
70. Gullberg U, et al. Biosynthesis, processing and sorting of neutrophil proteins: insight into neutrophil granule development. *Eur J Haematol.* 1997; 58(3):137–53. [PubMed: 9150707]
71. Johnson SR, et al. Characterization of a catalase-deficient strain of *Neisseria gonorrhoeae*: evidence for the significance of catalase in the biology of *N. gonorrhoeae*. *Infect Immun.* 1993; 61(4):1232–8. [PubMed: 8454325]
72. Tseng HJ, et al. Accumulation of manganese in *Neisseria gonorrhoeae* correlates with resistance to oxidative killing by superoxide anion and is independent of superoxide dismutase activity. *Mol Microbiol.* 2001; 40(5):1175–86. [PubMed: 11401721]
73. Stohl EA, Seifert HS. *Neisseria gonorrhoeae* DNA recombination and repair enzymes protect against oxidative damage caused by hydrogen peroxide. *J Bacteriol.* 2006; 188(21):7645–51. [PubMed: 16936020]
74. Skaar EP, et al. The outer membrane localization of the *Neisseria gonorrhoeae* MsrA/B is involved in survival against reactive oxygen species. *Proc Natl Acad Sci U S A.* 2002; 99(15):10108–13. [PubMed: 12096194]
75. Seib KL, et al. Investigation of oxidative stress defenses of *Neisseria gonorrhoeae* by using a human polymorphonuclear leukocyte survival assay. *Infect Immun.* 2005; 73(8):5269–72. [PubMed: 16041054]

76. Simons MP, et al. Interactions of *Neisseria gonorrhoeae* with adherent polymorphonuclear leukocytes. *Infect Immun*. 2005; 73(4):1971–7. [PubMed: 15784537]
77. Frangipane JV, Rest RF. Anaerobic growth of gonococci does not alter their Opa-mediated interactions with human neutrophils. *Infect Immun*. 1992; 60(5):1793–9. [PubMed: 1563766]
78. Casey SG, et al. *Neisseria gonorrhoeae* survive intraleukocytic oxygen-independent antimicrobial capacities of anaerobic and aerobic granulocytes in the presence of pyocin lethal for extracellular gonococci. *Infect Immun*. 1986; 52(2):384–9. [PubMed: 2870986]
79. Stohl EA, et al. The transcriptome response of *Neisseria gonorrhoeae* to hydrogen peroxide reveals genes with previously uncharacterized roles in oxidative damage protection. *Mol Microbiol*. 2005; 58(2):520–32. [PubMed: 16194237]
80. Stohl EA, et al. *Neisseria gonorrhoeae* metalloprotease NGO1686 is required for full piliation, and piliation is required for resistance to H₂O₂- and neutrophil-mediated killing. *MBio*. 2013; 4(4):e00399–13. [PubMed: 23839218]
81. Shafer WM, et al. Antigonococcal activity of human neutrophil cathepsin G. *Infect Immun*. 1986; 54(1):184–8. [PubMed: 3093384]
82. Shafer WM, et al. Molecular mechanism for the antigonococcal action of lysosomal cathepsin G. *Mol Microbiol*. 1990; 4(8):1269–77. [PubMed: 2126324]
83. Jerse AE, et al. A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection. *Infect Immun*. 2003; 71(10):5576–82. [PubMed: 14500476]
84. Kandler JL, et al. The MisR Response Regulator Is Necessary for Intrinsic Cationic Antimicrobial Peptide and Aminoglycoside Resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother*. 2016; 60(8):4690–700. [PubMed: 27216061]
85. West SE, Sparling PF. Response of *Neisseria gonorrhoeae* to iron limitation: alterations in expression of membrane proteins without apparent siderophore production. *Infect Immun*. 1985; 47(2):388–94. [PubMed: 3155708]
86. Noinaj N, et al. TonB-dependent transporters: regulation, structure, and function. *Annu Rev Microbiol*. 2010; 64:43–60. [PubMed: 20420522]
87. Agarwal S, et al. The gonococcal Fur-regulated *thpA* and *thpB* genes are expressed during natural mucosal gonococcal infection. *Infect Immun*. 2005; 73(7):4281–7. [PubMed: 15972520]
88. Bao Y, et al. Mitochondria regulate neutrophil activation by generating ATP for autocrine purinergic signaling. *J Biol Chem*. 2014; 289(39):26794–803. [PubMed: 25104353]
89. Attack JM, et al. A role for lactate dehydrogenases in the survival of *Neisseria gonorrhoeae* in human polymorphonuclear leukocytes and cervical epithelial cells. *J Infect Dis*. 2014; 210(8):1311–8. [PubMed: 24737798]
90. Boskey ER, et al. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum Reprod*. 2001; 16(9):1809–13. [PubMed: 11527880]
91. Britigan BE, et al. Phagocyte-derived lactate stimulates oxygen consumption by *Neisseria gonorrhoeae*. An unrecognized aspect of the oxygen metabolism of phagocytosis. *J Clin Invest*. 1988; 81(2):318–24. [PubMed: 3123517]
92. Fu HS, et al. Oxidant stress in *Neisseria gonorrhoeae*: adaptation and effects on L-(+)-lactate dehydrogenase activity. *Infect Immun*. 1989; 57(7):2173–8. [PubMed: 2543633]
93. Ragland SA, Criss AK. From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathog*. 2017; 13(9):e1006512. [PubMed: 28934357]
94. Humbert MV, et al. Structure of the *Neisseria* Adhesin Complex Protein (ACP) and its role as a novel lysozyme inhibitor. *PLoS Pathog*. 2017; 13(6):e1006448. [PubMed: 28662181]
95. Ragland SA, et al. *Neisseria gonorrhoeae* employs two protein inhibitors to evade killing by human lysozyme. *PLoS Pathog*. 2018; 14(7):e1007080. [PubMed: 29975775]
96. Zielke RA, et al. SliC is a surface-displayed lipoprotein that is required for the anti-lysozyme strategy during *Neisseria gonorrhoeae* infection. *PLoS Pathog*. 2018; 14(7):e1007081. [PubMed: 29975784]
97. Dillard JP, Hackett KT. Mutations affecting peptidoglycan acetylation in *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Infect Immun*. 2005; 73(9):5697–705. [PubMed: 16113287]

98. Cianciotto NP, Fields BS. *Legionella pneumophila mip* gene potentiates intracellular infection of protozoa and human macrophages. *Proc Natl Acad Sci U S A*. 1992; 89(11):5188–91. [PubMed: 1594630]
99. Reimer A, et al. Inhibitors of macrophage infectivity potentiator-like PPIases affect neisserial and chlamydial pathogenicity. *Int J Antimicrob Agents*. 2016; 48(4):401–8. [PubMed: 27516227]
100. Morishima T, et al. Neutrophil differentiation from human-induced pluripotent stem cells. *J Cell Physiol*. 2011; 226(5):1283–91. [PubMed: 20945397]

GLOSSARY

Antigenic Variation	Alteration of the composition of bacterial surface components. This can occur by DNA recombination as for pilin antigenic variation, or by phase variation (see below) of biosynthetic enzymes as for LOS. This variation leads to a heterogenic phenotypic population and is thought to be a key strategy to avoid immune recognition.
Complement	a cohort of soluble proteins in the immune system that deposit on the surface of microorganisms and aid in their phagocytic recognition and direct elimination.
Lipid A	Hydrophobic lipid component of LOS, also known as endotoxin.
LOS	Lipooligosaccharide is the <i>Neisserial</i> version of lipopolysaccharide. Like LPS, it is found in the outer leaflet of the outer membrane of Gc and consists of a membrane-anchored lipid A and surface-exposed sugars. Unlike LPS, LOS does not have a long O-antigen.
NADPH oxidase	Membrane bound enzyme complex found in neutrophils that produces superoxide and other reactive oxygen species.
NET	Neutrophil extracellular traps have a DNA backbone that is decorated with antimicrobial proteins and peptides. They can capture extracellular microbes and kill them, or limit their growth.
NLR	Nucleotide-binding oligomerization domain-like receptors are cytosolic pattern recognition receptors that recognize conserved microbial components and subsequently initiate signaling events that result in proinflammatory cytokine production.
Opa	Opacity associated proteins that are involved in intimate attachment of Gc to human tissues and cell types, including neutrophils. Opa proteins bind to CEACAM receptors as well as heparin sulfate proteoglycans; the former is crucial for the interactions of unopsonized Gc with neutrophils.

Opsonization	Process in which bacteria are marked by antibodies or complement for phagocytosis.
Phase Variation	On/off variation of protein expression, which in Gc is primarily through slipped-strand mispairing of DNA repeat elements in genes or their promoter elements. Phase-variable components of Gc include Opa proteins and some of the sugar glycosyltransferases for LOS. This variation leads to a heterogenic phenotypic population and is thought to be a key strategy to avoid immune recognition.
Porin	Outer membrane beta barrel proteins that act as nonselective pores in bacterial membranes, allowing molecules to passively diffuse into the periplasm. Gc has one porin, PorB, with at least 30 alleles that are continually evolving by genetic exchange and mutation.
ROS	Reactive oxygen species made by neutrophils include superoxide, hydroxyl radical, hydrogen peroxide, and hypochlorous acid. ROS are microbicidal but can also cause damage to tissues. Additionally, ROS are now recognized as initiators of cell-cell signaling events.
TGF-β	Transforming growth factor- β is a multifunctional cytokine that is involved in the downstream activation of immune cells
TLR	Toll-like receptors are found on the host cell surface and on intracellular organelles. They recognize conserved microbial components and subsequently initiate signaling events that result in proinflammatory cytokine production.

HIGHLIGHTS

- The mechanism by which neutrophils bind and phagocytose Gc dictates the extent of neutrophil activation (phagolysosome formation) and consequent bacterial survival.
- The neutrophil oxidative burst has a limited, if any, role in the ability of neutrophils to combat Gc.
- Gonococcal surface-exposed features such as Opa proteins, pili, and LOS affect neutrophil activation and bacterial susceptibility to neutrophil antimicrobial components. This is also true for PG in the bacterial cell envelope.
- Gc expresses surface components that contribute to defense from neutrophil antimicrobial components.
- Gc can acquire nutrients from neutrophils, including from neutrophil proteins that sequester essential metals.

OUTSTANDING QUESTIONS

- How does innate immune receptor recognition of neisserial pathogen-associated molecular patterns, such as LOS or PG, affect neutrophil activation and antigenococcal activity?
- How are Opa- bacteria internalized into neutrophils?
- How does the CEACAM binding pattern of Opa proteins affect neutrophil activation and antimicrobial activity?
- How does Gc induce NETs?
- What gene regulatory networks contribute to Gc survival from neutrophils?
- What are the fates of Gc and neutrophils after co-culture, and do they change depending on the factors expressed by the bacteria (such as Opa proteins)?

Figure 1. Gram stain of a gonorrheal exudate from an individual with uncomplicated urethral gonorrhea. Exudates contain neutrophils with associated Gc, which are found both inside (black arrows) and attached (blue arrows) to the cells.

Figure 2.

Gc survival after human neutrophil challenge (**Key Figure**). As an obligate human pathogen, Gc has evolved an infection strategy where it stimulates the local recruitment of neutrophils and survives in their presence. Gc has two general ways to survive from neutrophils. First, it expresses defenses against neutrophil antimicrobial components that are found intracellularly in phagosomes and/or extracellularly, free or in neutrophil extracellular traps (NETs). Second, it modulates the antimicrobial pathways of neutrophils. Gc limits opsonophagocytosis by varying antigenic surface components to avoid antibody-mediated recognition and limiting complement deposition (not shown). Gc phase-varies surface-exposed Opa proteins to modulate the efficiency of non-opsonic phagocytosis and to affect phagosome-granule fusion and consequent phagolysosome formation. Opsonic and non-opsonic interactions also affect the ability of neutrophils to assemble NADPH oxidase and mount the oxidative burst. Finally, Gc produces a nuclease to degrade NETs and survive extracellularly in association with neutrophils. Together, these strategies enable a subset of Gc to survive this immune response, which sustains within-host infection and enhances potential for transmission to new individuals. CEACAM, carcinoembryonic antigen-related cellular adhesion molecule; ROS, reactive oxygen species.

Figure 3.

Phagocytosis of Gc by neutrophils. Left: Gc Opa proteins bind to neutrophil CEACAMs, which drive nonopsonic phagocytosis of Gc. Right: Factor H and C4 binding protein (C4BP) binding to Gc porin and other Gc targets (not depicted) prevents the binding of iC3b and subsequent phagocytosis via the CR3 integrin heterodimer. Sialylation of LOS enhances the deposition of Factor H on porin. Not shown: Opa proteins, lipooligosaccharide (LOS), and pili vary on the Gc surface, which can prevent antibody-mediated recognition and subsequent opsonic phagocytosis.

Figure 4.

Maturation of the Gc phagosome in neutrophils. Left, Opa⁺ bacteria that interact with CEACAMs are directed into a mature phagolysosome which has fused with both secondary and primary granules. The majority of Gc that are immediately directed into phagolysosomes are killed. Similar observations are made for IgG-opsonized bacteria that interact with Fc γ receptors (not depicted). Right, in contrast, Opa⁻ bacteria are phagocytosed into a phagosome that is delayed for fusion with primary granules. The majority of Gc in immature phagosomes survive. Serum (complement)-opsonized Gc similarly are found in immature phagosomes in neutrophils, though they are phagocytosed more quickly than unopsonized bacteria (not depicted).

Figure 5.

Defenses of *Gc* against neutrophil antimicrobial components. In either the intracellular or extracellular environment, *Gc* is subjected to a multitude of antimicrobial neutrophil components with which it must contend. Depicted here for simplicity is phagosomally-localized *Gc*, although the location where bacterial defenses exert their effects may not be known at this time. Also depicted are selected bacterial defenses that contribute to survival from NETs. Defenses that contribute to *Gc* survival from neutrophil antimicrobial components include the metalloprotease Mpg, the LOS-modifying enzyme LptA, the peptidoglycan lytic transglycosylases LtgA and LtgD, the bacterial nucleoid-like protein RecN, and the peptidyl-prolyl isomerase MIP. The lysozyme inhibitors NGO1063/SliC and NGO1981 and the PacA-PacB peptidoglycan O-acetyltransferase system also enable defense from killing by neutrophils (not shown). For nutrient acquisition, *Gc* exploit neutrophil lactate as a carbon source via lactate dehydrogenase enzymes (Ldh). Extracellularly, *Gc* must also contend with NETs that can kill bacteria with CAMPs as well as through sequestration of nutrients. In addition to defenses like LptA, *Gc* have a nuclease that can break down NETs and acquire calprotectin-bound zinc via TdfH.

Table 1

Selected contents of neutrophil granules

GRANULES	CATEGORY	CONTENTS
Primary	<i>Antimicrobial proteins</i>	α -defensins
		Bacteriocidal/Permeability Increasing Protein
		Azurocidin
	<i>ROS production</i>	Myeloperoxidase
	<i>Serine Proteases</i>	Cathepsin G
		Proteinase
		Neutrophil Elastase
	<i>Degradative enzymes</i>	Lysozyme
Secondary	<i>Antimicrobial proteins</i>	Lactoferrin
		LL-37
	<i>Degradative enzymes</i>	Collagenase
		Gelatinase
		Lysozyme
	<i>ROS production</i>	Cytochrome b558
Tertiary	<i>Degradative enzymes</i>	Gelatinase
		Lysozyme
	<i>ROS production</i>	Cytochrome b558