Location, Location, Location—Commensalism, Damage and Evolution of the Pathogenic *Neisseria*

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Abstract

The 10 human-restricted *Neisseria species* all colonize mucosal surfaces, but show a spectrum of pathogenicity. The commensal *Neisseria* do not normally cause pathology, while the two pathogenic species, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, straddle the border between commensalism and pathogenicity. Why the pathogenic *Neisseria* continue to mediate host damage after thousands of years of co-evolution with their human host, and why the commensal species have not acquired the ability to damage the host, if this capability provides a selective advantage, is not understood. One way the pathogenic species are different from the commensal species is by their ability to induce PMN inflammation, which is dependent on the site of colonization. I discuss how the site of colonization dictates whether copious inflammation occurs with both pathogenic species. I put forth a model that posits that an ancestor of both pathogenic species changed colonization site from the oral cavity to the genital tract of a human or humanoid and had to evolve multiple, new traits — to induce PMN inflammation and avoid adaptive immunity — to allow efficient sexual transmission. This model predicts that PMN inflammation produces the serious sequelae of gonorrhea and increases the probability that *N. meningitidis* might exit the oral cavity to produce systemic disease. In both cases, the pathology produced by these host-adapted species is an unintended by product of the inflammation but host damage does not provide any selective advantage for these organisms.

There are 10 *Neisseria* species that colonize humans, and five of these are only found within humans and are not isolated from other animals or environmental sources [1]. While most of these organisms colonize animals without causing overt pathology, two species have evolved to cause disease in humans and the sites of colonization have proven to be important to their pathogenicity. The pathogenic *Neisseria*, *Neisseria gonorrhoeae* (the gonococcus) and *Neisseria meningitidis* (the meningococcus), are closely related, human-restricted, organisms that could be considered subspecies rather than separate species [2]. *N. meningitidis* usually colonizes the nasal pharynx where it does not cause any overt pathology and can be considered a normal part of the respiratory tract microbiome. However, the meningococcus can leave the normal niche to cause serious disease [3]. The commensal *Neisseria* colonize several different locations within the upper respiratory tract [4], but do not have enhanced ability to leave the normal sites of colonization, and perhaps have reduced ability to survive systemically [5]. This barrier to systemic spread is partially due to their sensitivity to complement [6]. The *Neisseria* are all naturally competent for DNA transformation, and there is evidence for gene flow between different *Neisseria* species [7]. *N. gonorrhoeae* usually colonizes the urogenital tract of men and women, is mainly sexually transmitted, and is more often inflammatory than the other *Neisseria* species [8]. In this Perspective, I will discuss the different ways the human-restricted *Neisseria* interact with their host and how the sites of colonization can influence host responses, and propose a hypothesis that altering the site of colonization provided the selective pressure to allow for sexual transmission that allowed the pathogenic species to arise.

N. meningitidis is the main cause of bacterial meningitis in teenagers and young adults and is one

of the many bacterial species that cause meningitis in babies [3]. *N. meningitidis* also can also cause the disseminated disease, meningococcemia. While the organism may transit from the bloodstream directly to the central nervous system, many cases of meningococcal meningitis do not show meningococcemia, suggesting that there may be other routes from oral cavity to the meninges [9]. In most people, *N. meningitidis* stays within the nasal pharynx and does not induce obvious immune responses no host damage. Carriage of meningococci in populations usually hovers around 10% of the population and is increased in young adults by smoking and/or frequenting bars and parties [10–13]. While the meningococcus leaves the respiratory tract infrequently, it has a much higher probability to becoming systemic than the commensals. Why *N. meningitidis* has acquired increased ability to establish systemic infection, a dead end route of infection, is not known but presumably relies on factors that provide other selective advantages to this organism. Why there are outbreaks of invasive meningococcal disease is also not known, but the rates of invasive disease are not always predicted by carriage rates [14]. There are environmental factors (e.g., young adults from different location in close contact or the dry season in sub-Saharan Africa) that correlate with increased invasive disease and clones that show higher probability of producing invasive disease [15]. It is notable that meningococcal colonization has been suggested to be of shorter duration than that of the commensal *Neisseria* [15], suggesting that *N. meningitidis* may be more often recognized by the host as foreign and therefore cleared. It is not known what host factors are responsible for clearance, but data from a mouse model suggest that it is possibly due to innate immune responses [16]. Epidemiology data also suggest that the meningococcus may transmit more frequently than the commensal *Neisseria* to maintain their colonization levels in populations [15]. There are prospective, longitudinal studies occurring presently that may provide support for these ideas [17].

It is likely that more than one genetic attribute contributes to *N. meningitidis* invasive disease since there has not been a single trait identified that correlates with invasiveness, nor has this phenotype been transferred to the closely related commensal *Neisseria*. Regardless of the reasons behind the increased ability of *N. meningitidis* to leave and survive outside of the normal sites of colonization, it is this ability that makes this species a deadly pathogen. If *N. meningitidis* never transited to the blood stream or meninges, it would be classified as a commensal organism, but the fact that it can cause morbidity and mortality at a detectable frequency makes it pathogenic.

The situation for *N. gonorrhoeae* as an organism that can elicit different types of host responses is more complicated. Gonococcal genital colonization produces symptomatic gonorrhea when sufficient numbers of polymorphonuclear leukocytes (PMNs) are recruited to the site of colonization to produce the so-called purulent exudate consisting of PMNs and gram-negative diplococci [8]. There are epidemiology data that support the hypothesis that infection of women is usually asymptomatic, while infection in men is usually symptomatic and often results in painful urination [18,19]. This viewpoint has recently been challenged because detection of an exudate in women is more difficult and it may be arbitrary to define symptomatic infections by an obvious purulent exudate [8,20,21]. It is unknown whether PMNs are recruited to the sites of infection when an obvious purulent exudate is not observed. Mouse models of genital infection show that PMN recruitment is common during gonococcal colonization [22,23]. Rates of gonococcal rectal and oral colonization are increasing, but colonization of these locations does not usually result in a purulent exudate [24]. Whether there are PMNs recruited to these sites of colonization at lower numbers is also not known. Colonization of the conjunctiva can occur during birth, by autoinoculation, or during laboratory inoculation and is very inflammatory [25,26]. Finally, PMN and bacterial-derived products can damage host tissues to cause pelvic inflammatory disease [27,28]. Therefore, the site of gonococcal colonization strongly influences the magnitude of the host response, and the sensitivity of the tissues being colonized to PMN inflammation dictates the level of pathology. In the case of *N. gonorrhoeae*, non-inflammatory colonization is not considered a commensal state but instead is an asymptomatic infection.

In rare cases, the gonococcus can also leave the genital tract to cause disseminated gonococcal disease (DGI), with the possible associated sequelae of infectious arthritis, rash, endocarditis, or meningitis [29]. The probability of DGI is low, and similarly to the meningococcus, certain isolates possess a higher probability of causing DGI, but no single genetic locus has been identified. Colonizing other sites, for example, the epididymis, joints, or heart can also result in host damage presumably due to toxic bacterial or PMN products.

Whole-genome sequence analysis of presumed gonococcal isolates from male urethritis cases revealed that some *N. meningitidis* clones can cause urethritis during urogenital colonization that is indistinguishable from that caused by *N. gonorrhoeae* [30,31]. Interestingly, these meningococcal urethritis producing clones lost expression of the capsule and acquired a few gonococcal genes that help with growth in reduced oxygen, presumably to help them survive in the genital tract [31]. It has not been determined whether the urethral inflammation of these meningococcal clones is related to the acquisition of the gonococcal genes or

is solely dependent on the site of colonization. Meningococcal urethritis was reported as early as 1942 [32], but it is uncertain how long *N. meningitidis* clones have been colonizing the urogenital tract and were misdiagnosed as gonorrhea. Regardless of the natural history of these infections, these recent observations indicate that gene flow between the species is common and can influence where colonization can occur. The fact that both the gonococcus and meningococcus are inflammatory in the genital tract, but neither is as inflammatory in the upper respiratory tract supports the idea that the location of colonization influences the strength of the inflammatory signals elicited by these organisms.

Phylogenetic analyses of genomic sequences suggest that the human-restricted *Neisseria* all arose from a common ancestor [33], suggesting that a single organism colonized an early human or humanoid oral cavity, and then lost the ability to live outside of the human body. The fact that *Neisseria* DNA has been detected in dental calculus samples from Neanderthal samples supports this model [34]. This proposed lineage raises the question of what might have occurred to provide a trait or traits to the pathogenic *Neisseria,* which are not present in the commensal *Neisseria,* but would not provide a strong enough selective advantage to ensure horizontal gene transfer to the other commensal *Neisseria* species. Indeed, there are many factors carried by the pathogenic species that are not found in the commensal species [35]. These include the three complex antigenic variation systems, the large number of phase variable genes, the Maf immunity system, the IgA protease, the TdfF iron uptake system, and diploid, homozygous chromosomes [35]. There must have been multiple steps required to evolve these systems, including gene duplications, extensive modification of DNA sequences, and alterations in gene expression and function. These many genetic changes would have taken a long time to evolve, and also make it impossible to transfer the pathogenic potential to the commensal *Neisseria*, and these multi-genic changes also might make reversion of the meningococcal to a commensal state more difficult.

Lauren Prister (nee Priniski) and I have proposed that that the pathogenic *Neisseria* ancestor arose from a commensal progenitor within humans by acquiring the ability to induce PMN inflammation, while evolving ways to resist PMN killing mechanisms and avoiding adaptive immunity [35]. In this model, the common ancestor of the pathogenic *Neisseria* was a commensal organism that switched its main site of colonization from the oral cavity to the genital tract. Alison Criss and I have proposed that the recruitment of PMNs to the genital tract is beneficial to the gonococcus [36]. PMNs may allow more efficient transmission, particularly from women to men, since there is no obvious way for the bacterium to enter the male urethra. This hypothesis proposes that PMNs carry the gonococcus between women and men using a gradient of seminal plasma as a chemotactic substance. I propose that this strong selective pressure to allow for efficient sexual transmission, in the progenitor organism, was the primary reason for the evolution of the pathogenic *Neisseria*. In addition, if adapting to this new niche selected for multiple mutations/traits in this locale, the presence of multiple changes would have prevented the progenitor or the modern day pathogenic *Neisseria* from transferring these traits to the commensal *Neisseria* [35]. Moreover, many of these mutations would require compensating mutations to maintain fitness and these would prevent reversion of these traits in the meningococcus, if they indeed do not provide a selective advantage in the oral cavity. Thus, we propose that it was this change of colonization location that provided the strong selective pressure to evolve different capabilities for the pathogenic progenitor. This model is based on several assumptions, and there are many other reasonable evolutionary paths that might have occurred including evolution of the pathogenic *Neisseria* in a different animal and evolution of one or more commensal *Neisseria* from the pathogenic progenitor. I believe our model fits the available data but will require more in-depth investigation into the evolutionary relationships between the *Neisseria* species and more basic and epidemiology studies into mechanisms promoting *Neisseria* transmission, colonization, and interactions in innate responses.

This discussion of the dual lifestyles of the pathogenic *Neisseria* is complicated by our inadequate definitions of what constitutes commensalism and pathogenesis. A good definition of a commensal organism is one that when colonizing a normal location does not elicit a vigorous and sustained host response. However, it is clear that breaches in barriers, immune dysregulation, and incorrect colonization can produce serious pathology from a number of different commensal organisms. Is the ability to induce inflammation a sufficient definition for a pathogen? If so, then the gonococcus fits nicely within this classification of a pathogen, but the meningococcus does not, since it does not usually elicit inflammation. It should be noted that all of these organisms have been co-evolving with their human host for a long time and therefore should have lowered their pathogenic potential. Therefore, the human-restricted *Neisseria* do not fit nicely into strict definitions of commensalism or pathogenesis. It is, however, clear that the sites of colonization (i.e., location) of the *Neisseria* influence whether they act more as commensal or pathogenic organisms and the type of host responses that are elicited. We also suspect that the change of location of colonization of the pathogenic ancestor was necessary to provide strong selection for the development of new traits necessitated by the need for efficient sexual

transmission. It will be difficult to test many of the hypotheses presented here, but it is worth considering these ideas for these and other host-restricted organisms.

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PMN, polymorphonuclear leukocyte; DGI, disseminated gonococcal disease.

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