Neisseria meningitidis scanning EM

Introduction

The family Neisseriaceae consists of Gram-negative aerobic bacteria from fourteen genera (Bergey's 2001), including Neisseria, Chromobacterium, Kingella, and Aquaspirillum. The genus Neisseria contains two important human pathogens, N. gonorrhoeae and N. meningitidis. N. gonorrhoeae causes gonorrhea, and N. meningitidis is the cause of meningococcal meningitis. N. gonorrhoeae infections have a high prevalence and low mortality, whereas N. meningitidis infections have a low prevalence and high mortality.

Neisseria gonorrhoeae infections are acquired by sexual contact and usually affect the mucous membranes of the urethra in males and the endocervix and urethra in females, although the infection may disseminate to a variety of tissues. The pathogenic mechanism involves the attachment of the bacterium to nonciliated epithelial cells via pili (fimbriae) and the production of lipopolysaccharide endotoxin. Similarly, the lipopolysaccharide of Neisseria meningitidis is highly toxic, and it has an additional virulence factor in the form of its antiphagocytic capsule. Both pathogens produce IgA proteases which promote virulence. Many normal individuals may harbor Neisseria meningitidis in the upper respiratory tract, but Neisseria gonorrhoeae is never part of the normal flora and is only found after sexual contact with an infected person (or direct contact, in the case of infections in the newborn).

In the vocabulary of the public health and medical microbiologist, N. gonorrhoeae is often referred to as the "gonococcus", while N. meningitidis is known as the "meningococcus" and one form of the disease it causes is called meningococcemia.
*Neisseria gonorrhoeae* is a Gram-negative coccus, 0.6 to 1.0 µm in diameter, usually seen in pairs with adjacent flattened sides (Figure 1 Left and Fig 2 below). The organism is frequently found intracellularly in polymorphonuclear leukocytes (neutrophils) of the gonorrhea pustular exudate (Figure 1 Right). Fimbriae, which play a major role in adherence, extend several micrometers from the cell surface (Figure 2 below).

*N. gonorrhoeae* possesses a typical Gram-negative outer membrane composed of proteins, phospholipids, and lipopolysaccharide (LPS). However, neisserial LPS is distinguished from enteric LPS by its highly-branched basal oligosaccharide structure and the absence of repeating O-antigen subunits. For these reasons, neisserial LPS is referred to as *lipooligosaccharide* (LOS). The bacterium characteristically releases outer membrane fragments called "blebs" during growth. These blebs contain LOS and probably have a role in pathogenesis if they are disseminated during the course of an infection.

*N. gonorrhoeae* is a relatively fragile organism, susceptible to temperature changes, drying, uv light, and other environmental conditions. Strains of *N. gonorrhoeae* are variable in their cultural requirements so that media containing hemoglobin, NAD, yeast extract and other supplements are needed for isolation and growth of the organism. Cultures are grown at 35-36 degrees in an atmosphere of 3-10% added CO₂.

**Infections caused by *N. gonorrhoeae***

The disease gonorrhea is a specific type of urethritis that practically always involves mucous membranes of the urethra, resulting in a copious discharge of pus, more apparent in the male than in the female. The first usage of the term "gonorrhea", by Galen in the second century, implied a "flow of seed". For centuries thereafter, gonorrhea and syphilis were confused, resulting from the fact that the two diseases were often present together in infected individuals. Paracelsus (1530) thought that gonorrhea was an early symptom of syphilis. The confusion was further heightened by the classic blunder of English physician John Hunter, in 1767. Hunter intentionally inoculated himself with pus from a patient with symptoms of gonorrhea and wound up giving himself syphilis! The causative agent of gonorrhea, *Neisseria gonorrhoeae*, was first described by A. Neisser in 1879 in the pustular exudate of a
case of gonorrhea. The organism was grown in pure culture in 1885, and its etiological relationship to human disease was later established using human volunteers in order to fulfill the experimental requirements of Koch's postulates.

Gonorrhea is generally limited to superficial mucosal surfaces lined with columnar epithelium. The areas most frequently involved are the urethra, cervix, rectum, pharynx, and conjunctiva. Squamous epithelium, which lines the adult vagina, is not susceptible to infection by the *N. gonorrhoeae*. However, the prepubescent vaginal epithelium, which has not been keratinized under the influence of estrogen, may be infected. Hence, gonorrhea in young girls may present as vulvovaginitis. Mucosal infections are usually characterized by a purulent discharge.

Uncomplicated gonorrhea in the adult male is an inflammatory and pyogenic infection of the mucous membranes of the anterior urethra. The most common symptom is a discharge that may range from a scanty, clear or cloudy fluid to one that is copious and purulent. Dysuria (difficulty in urination) is often present. Inflammation of the urethral tissues results in the characteristic redness, swelling, heat, and pain in the region. There is intense burning and pain upon urination.

Endocervical infection is the most common form of uncomplicated gonorrhea in women. Such infections are usually characterized by vaginal discharge and sometimes by dysuria. About 50% of women with cervical infections are asymptomatic. Asymptomatic infections occur in males, as well. Males with asymptomatic urethritis are an important reservoir for transmission and are at increased risk for developing complications. Asymptomatic males and females are a major problem as unrecognized carriers of the disease, which occurs in the U.S. at an estimated rate of over one million cases per year.

In the male, the organism may invade the prostate resulting in prostatitis, or extend to the testicles resulting in orchitis. In the female, cervical involvement may extend through the uterus to the fallopian tubes resulting in salpingitis, or to the ovaries resulting in ovariitis. As many as 15% of women with uncomplicated cervical infections may develop pelvic inflammatory disease (PID). The involvement of testicles, fallopian tubes or ovaries may result in sterility. Occasionally, disseminated infections occur. The most common forms of disseminated infection are a dermatitis-arthritis syndrome, endocarditis and meningitis.

Rectal infections (proctitis) with *N. gonorrhoeae* occur in about one-third of women with cervical infection. They most often result from autoinoculation with cervical discharge and are rarely symptomatic. Rectal infections in homosexual men usually result from anal intercourse and are more often symptomatic. Partners must be treated as well to avoid reinfection.

Ocular infections by *N. gonorrhoeae* can have serious consequences of corneal scarring or perforation. Ocular infections (ophthalmia neonatorum) occur most commonly in newborns who are exposed to infected secretions in the birth canal. Part of the intent in adding silver nitrate or an antibiotic to the eyes of the newborn is to prevent ocular infection by *N. gonorrhoeae*.

**Pathogenesis**

Gonorrhea in adults is almost invariably transmitted by sexual intercourse. The bacteria adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is mediated through fimbriae and opa (P.II) proteins. Although nonspecific factors such as surface charge and hydrophobicity may play a role. Fimbriae undergo both phase and antigenic variation. The bacteria attach only to microvilli of nonciliated columnar epithelial cells. Attachment to ciliated cells does not occur.

Most of the information on bacterial invasion comes from studies with tissue culture cells and human fallopian tube organ culture. After the bacteria attach to the nonciliated epithelial cells of the fallopian tube, they are surrounded by the microvilli, which draw them to the surface of the mucosal cell. The bacteria enter the epithelial cells by a process called parasite-directed endocytosis. During endocytosis the membrane of the mucosal cell retracts and pinches off a membrane-bound vacuole that contains the bacteria. The vacuole is transported to the base of the cell, where the bacteria are released by exocytosis
into the subepithelial tissue. The neisseriae are not destroyed within the endocytic vacuole, but it is not clear whether they actually replicate in the vacuoles as intracellular parasites.

A major porin protein, **P.I (Por)**, in the outer membrane of the bacterium is thought to be the invasin that mediates penetration of a host cell. Each *N. gonorrhoeae* strain expresses only one type of Por; however, there are several variations of Por that partly account for different antigenic types of the bacterium.

**Neisseria gonorrhoeae** can produce one or several outer membrane proteins called Opa (**P.II**) proteins. These proteins are subject to phase variation and are usually found on cells from colonies possessing a unique opaque phenotype called **O+**. At any particular time, the bacterium may express zero, one, or several different Opa proteins, and each strain has 10 or more genes for different Opas.

**Rmp (**P.III**)** is an outer membrane protein found in all strains of *N. gonorrhoeae*. It does not undergo antigenic variation and is found in a complex with Por and LOS. It shares partial homology with the OmpA protein of *Escherichia coli*. Antibodies to Rmp, induced either by a neisserial infection or by colonization with *E. coli*, tend to block bactericidal antibodies directed against Por and LOS. In fact, anti-Rmp antibodies may increase susceptibility to infection by *N. gonorrhoeae*.

During infection, bacterial lipooligosaccharide (LOS) and peptidoglycan are released by autolysis of cells. Both bacterial polysaccharides activate the host alternative complement pathway, while LOS also stimulates the production of tumor necrosis factor (TNF) that causes cell damage. Neutrophils are immediately attracted to the site and feed on the bacteria. For unknown reasons, many gonococci are able to survive inside of the phagocytes, at least until the neutrophils themselves die and release the ingested bacteria.

Neisserial LOS has a profound effect on the virulence and pathogenesis of *N. gonorrhoeae*. The bacteria can express several antigenic types of LOS and can alter the type of LOS they express by some unknown mechanism. Gonococcal LOS produces mucosal damage in fallopian tube organ cultures and brings about the release of enzymes, such as proteases and phospholipases, that may be important in pathogenesis. Thus, gonococcal LOS appears to have an indirect role in mediating tissue damage. Gonococcal LOS is also involved in the resistance of *N. gonorrhoeae* to the bactericidal activity of normal human serum. Specific LOS oligosaccharide types are known to be associated with a serum-resistant phenotype of *N. gonorrhoeae*.

*N. gonorrhoeae* can utilize host-derived N-acetylneuraminic acid (sialic acid) to sialylate the oligosaccharide component of its LOS, converting a serum-sensitive organism to a serum-resistant one. Organisms with nonsialylated LOS are more invasive than those with sialylated LOS but organisms with sialylated LOS are more resistant to bactericidal effects of serum. There is also antigenic similarity between neisserial LOS and antigens present on human erythrocytes. This similarity to "self" may preclude an effective immune response to these LOS antigens by maintaining the immunotolerance of the host.

*N. gonorrhoeae* is highly efficient at utilizing transferrin-bound iron for in vitro growth; many strains can also utilize lactoferrin-bound iron. The bacteria bind only human transferrin and lactoferrin. This specificity is thought to be, in part, the reason these bacteria are exclusively human pathogens.

Strains of *N. gonorrhoeae* produce two distinct extracellular IgA1 proteases which cleave the heavy chain of the human immunoglobulin at different points within the hinge region. Split products of IgA1 have been found in the genital secretions of women with gonorrhea, suggesting that the bacterial IgA1 protease is present and active during genital infection. It is thought that the Fab fragments of IgA1 may bind to the bacterial cell surface and block the Fc-mediated functions other immunoglobulins.

Occasionally, as described above, invading *Neisseria gonorrhoeae* enter the bloodstream causing a Gram-negative bacteremia which may lead to a disseminated bacterial infection. Asymptomatic infections of the urethra or cervix usually serve as focal sources for bacteremia. Strains of *N. gonorrhoeae* that cause disseminated infections are usually resistant to complement and the serum bactericidal reaction. This accounts for their ability to persist in the bacteremia. In Gram-negative bacteremias of this sort, the
effect of bacterial endotoxin can be exacerbated by the lysis of bacterial cells which may simply liberate soluble LPS.

**Figure 3. Pathogenesis of uncomplicated gonorrhea according to Morse in Baron, Chapter 14, Neisseria, Branhamella, Moraxella and Eikenella**

**Virulence Factors**

Like the other pyogenic bacteria, *Neisseria gonorrhoeae* has a wide range of virulence determinants, although it does not produce any exotoxins. The first stages of infection, involving adherence and invasion, are mediated by surface components of the gonococci. The bacterium first attaches to epithelial cells by means of its fimbriae, specifically N-methylphenylalanine (Type 4) pili, the main subunit of which is PilE. After initial attachment, the bacteria enter a second stage of binding mediated by the outer membrane protein P.II (also known as Opa) which is needed for tight binding and invasion of epithelial cells. Also, P.II from one bacterium will bind to LOS of an adjacent bacterium, which allows for the construction of a microcolony which may be functionally analogous to a biofilm. However, the invasion of a cell involves a single bacterium, not whole microcolonies. *Neisseria gonorrhoeae* also produces an IgA1 protease that probably play a role in the colonization stage.

The outer membrane porin of *N. gonorrhoeae* P.I (also known as Por) is equivalent to the ompC and ompF porins of *E. coli* that are involved in the passage of solutes through the outer membrane. However, P.I apparently has a role in virulence that allows the gonococci to survive inside of phagocytes. Purified P.I has been shown to inhibit the ability of phagocytes to kill ingested bacteria.

The lipooligosaccharide (LOS) of the outer membrane is thought to be responsible for most of the symptoms of gonorrhea. Gonococcal LOS triggers an intense inflammatory response. Subsequent activation of complement, attraction and feeding by phagocytes, and the lysis of the phagocytes themselves, contributes to the purulent discharge. The local production of TNF, elicited by LOS, is
thought to be the main cause of damage to the fallopian tubes. In addition, in strains that cause systemic infection, LOS binds sialic acid from the serum forming a microcapsule of sialylated LOS, which allows the gonococci to resist the host immune response and serum bactericidal reaction.

Nonsialylated LOS and P.I (Por) on the bacterial surface are known to be effective targets for bactericidal antibodies. However, if antibodies produced against P.III (also known as Rmp) react with their antigenic site on the gonococcal surface, the effect is to block bactericidal antibodies against LOS and P.I and to protect the bacterium from complement-mediated lysis.

Finally, Neisseria gonorrhoeae has a well-developed iron acquisition system that permits it to extract iron from its host during growth, which is necessary to support bacterial invasion. Basically, the bacterium is able to form two transferrin receptors (Tbp1 and Tbp2) and one lactoferrin receptor (Lbp) in its outer membrane, which are induced under low-iron conditions, and which are able to directly extract iron from transferrin and lactoferrin, respectively. The proteins can also extract iron from heme and hemoglobin.

### Table 1. Surface components of *N. gonorrhoeae* that may play a role in virulence

<table>
<thead>
<tr>
<th>Designation</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>PilE</td>
<td>major fimbrial protein</td>
<td>initial binding to epithelial cells</td>
</tr>
<tr>
<td>P.II (Opa)</td>
<td>outer membrane protein</td>
<td>contributes to invasion</td>
</tr>
<tr>
<td>P.I (Por)</td>
<td>outer membrane porin</td>
<td>may prevent phagolysosome formation in neutrophils and/or reduce oxidative burst</td>
</tr>
<tr>
<td>LOS</td>
<td>outer membrane lipooligosaccharide</td>
<td>elicits inflammatory response, triggers release of TNF</td>
</tr>
<tr>
<td>P.III (Rmp)</td>
<td>outer membrane protein</td>
<td>elicits formation of ineffective antibodies that block that block bactericidal antibodies against P.I and LOS</td>
</tr>
<tr>
<td>Tbp1 and Tbp2</td>
<td>outer membrane receptors for transferrin</td>
<td>iron acquisition for growth</td>
</tr>
<tr>
<td>Lbp</td>
<td>outer membrane receptor for lactoferrin</td>
<td>iron acquisition for growth</td>
</tr>
</tbody>
</table>

### Host Defenses

Infection stimulates inflammation and a local immune (IgA) response. Inflammation focuses the host defenses but also becomes the pathology of the disease. It is not known whether the secretory immune response is protective. Serum antibodies also appear, and IgG and complement may be components of the inflammatory exudate. But whether the immune defenses provide much protection against reinfection has not been clearly shown. In any case, immunity is expected to be strain specific so that reinfection may occur.

Not everyone exposed to *N. gonorrhoeae* acquires the disease. This may be due to variations in the size or virulence of the inoculum, to natural resistance, or to specific immunity. A 50% infective dose (ID50) of
about 1,000 bacteria has been determined based on experimental urethral inoculation of male volunteers. No data is available for females.

Nonspecific factors have been implicated in natural resistance to gonococcal infection. In women, changes in the genital pH and hormones may increase resistance to infection at certain times of the menstrual cycle. Urine contains bactericidal and bacteriostatic components against *N. gonorrhoeae*. Factors in urine that may be important are pH, osmolarity, and the concentration of urea. The variability in the susceptibility of gonococcal strains to the bactericidal and bacteriostatic properties of urine is thought to be one of the reasons some males apparently do not develop a gonorrhea infection when exposed.

Most uninfected individuals have serum antibodies that react with gonococcal antigens. These antibodies probably result from colonization or infection by various Gram-negative bacteria that possess cross-reactive antigens. Such "natural antibodies" may be important in individual natural resistance or susceptibility to infection, but this has not been clearly demonstrated.

Infection with *N. gonorrhoeae* stimulates both mucosal and systemic antibodies to a variety of gonococcal antigens. Mucosal antibodies are primarily IgA and IgG. In genital secretions, antibodies have been identified that react with Por, Opa, Rmp and LOS. Vaccine trials have suggested that specific anti-fimbrial antibodies inhibit the fimbrial-mediated attachment of the homologous gonococcal strain. In general, the IgA response is brief and declines rapidly after treatment; IgG levels decline more slowly. Anti-Por antibodies apparently are bactericidal for the gonococcus. IgG that reacts with Rmp blocks the bactericidal activity of antibodies directed against Por and LOS. Genital infection with *N. gonorrhoeae* stimulates a serum antibody response against the LOS of the infecting strain. Disseminated gonococcal infection results in much higher levels of anti-LOS antibody than do genital infections.

Strains that cause uncomplicated genital infections usually are killed by normal human serum and are termed serum sensitive. This bactericidal activity is mediated by IgM and IgG antibodies that recognize sites on the LOS. Strains that cause disseminated infections are not killed by most normal human serum and are referred to as serum resistant. Resistance is mediated, in part, by IgA that blocks the IgG-mediated bactericidal activity of the serum. Serum from convalescent patients with disseminating infections contains bactericidal IgG to the LOS of the infecting strain.

Individuals with inherited complement deficiencies have a markedly increased risk of acquiring systemic neisserial infections and are subject to recurring episodes of systemic gonococcal and meningococcal infections, indicating that the complement system is important in host defense. Gonococci activate complement by both the classic and alternative pathways. Complement activation by gonococci leads to the formation of the C5b-9 complex (membrane attack complex) on the outer membrane. In normal human serum, similar numbers of C5b-9 complexes are deposited on serum-sensitive and serum-resistant organisms, but the membrane attack complex is not functional on serum-resistant organisms.

**Treatment**

The recommended treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolone plus an antibiotic (e.g., doxycycline or erythromycin) effective against possible coinfection with *Chlamydia trachomatis*. Sex partners should be referred and treated. The current CDC Treatment Guidelines recommend treatment of all gonococcal infections with antibiotic regimens effective against resistant strains. The recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin, or oflaxacin.

**Control**

There is no effective vaccine to prevent gonorrhea. Candidate vaccines consisting of PilE protein or Por are of little benefit. The development of an effective vaccine has been hampered by the lack of a suitable animal model and the fact that an effective immune response has never been demonstrated. Condoms are effective in preventing the transmission of gonorrhea.
The evolution of antimicrobial resistance in *N. gonorrhoeae* may ultimately affect the control of gonorrhea. Strains with multiple chromosomal resistance to penicillin, tetracycline, erythromycin, and cefoxitin have been identified in the United States and most other parts of the world. Sporadic high-level resistance to spectinomycin and fluoroquinolones has been reported. Penicillinase-producing strains of *N. gonorrhoeae* were first described in 1976. Five related ß-lactamase plasmids of different sizes have been identified. Their prevalence penicillin-resistant strains has increased dramatically in the United States since 1984.

Plasmid-mediated resistance of *N. gonorrhoeae* to tetracycline was first described in 1986 and has now been reported in most parts of the world. This resistance is due to the presence of the streptococcal tetM determinant on a gonococcal conjugative plasmid.

**Tailpiece**

The only natural host for *N. gonorrhoeae* is humans. Gonorrhea has all but disappeared in Scandinavia and several other European countries. However, the disease is very common in the United States. CDC estimates that more than 700,000 persons in the U.S. get new gonorrheal infections each year. Only about half of these infections are reported to CDC. In 2002, 351,852 cases of gonorrhea were reported to CDC. In the period from 1975 to 1997, the national gonorrhea rate declined, following the implementation of the national gonorrhea control program in the mid-1970s. After a small increase in 1998, the gonorrhea rate has decreased slightly since 1999. In 2002, the rate of reported gonorrheal infections was 125.0 per 100,000 persons.

Gonorrhea is transmitted almost exclusively by sexual contact. Any sexually active person can be infected with gonorrhea. In the United States, the highest reported rates of infection are among sexually active teenagers, young adults, and African Americans. Persons who have multiple sex partners are at highest risk. Rates of gonorrhea are higher in males and in minority and inner-city populations.

Gonorrhea is usually contracted from a sex partner who is either asymptomatic or has only minimal symptoms. It is estimated that the efficiency of transmission after one exposure is about 35 percent from an infected woman to an uninfected man and 50 to 60 percent from an infected man to an uninfected woman. More than 90 percent of men with urethral gonorrhea will develop symptoms within 5 days; fewer than 50 percent of women with genital gonorrhea will do so. Women with asymptomatic infections are at higher risk of developing pelvic inflammatory disease and disseminated gonococcal infection.

**Neisseria meningitides**

The bacterium *Neisseria meningitidis*, the *meningococcus*, is identical in its staining and morphological characteristics to *Neisseria gonorrhoeae*. However, at the ultrastructural level, *N. meningitidis* has a prominent antiphagocytic polysaccharide capsule. *N. meningitidis* strains are grouped on the basis of their capsular polysaccharides, into 12 serogroups, some of which are subdivided according to the presence of outer membrane protein and lipopolysaccharide antigens.

*Neisseria meningitidis* is usually cultivated in a peptone-blood base medium in a moist chamber containing 5-10% CO₂. All media must be warmed to 37 degrees prior to inoculation as the organism is extremely susceptible to temperatures above or below 37 degrees. This trait is rather unique among bacteria. Also, the organism tends to undergo rapid autolysis after death, both in vitro and in vivo. This accounts for the dissemination of lipopolysaccharide (endotoxin) during septicemia and meningitis.

The organism tends to colonize the posterior nasopharynx of humans, and humans are the only known host. Individuals who are colonized are carriers of the pathogen who can transmit disease to nonimmune individuals. The bacterium also colonizes the posterior nasopharynx in the early stages of infection prior to invasion of the meninges. Most individuals in close contact with a case of meningococcal meningitis become carriers of the organism. This carrier rate can reach 20 percent of the contact group before the first case is recognized, and may reach as high as 80 percent at the height of an epidemic.

**Structure and Classification**
The only distinguishing structural feature between *N. meningitidis* and *N. gonorrhoeae* is the presence of a polysaccharide capsule in the former. The capsule is antiphagocytic and is an important virulence factor.

Meningococcal capsular polysaccharides provide the basis for grouping the organism. Twelve serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W135). The most important serogroups associated with disease in humans are A, B, C, Y, and W135. The chemical composition of these capsular polysaccharides is known. The prominent outer membrane proteins of *N. meningitidis* have been designated class 1 through class 5. The class 2 and 3 proteins function as porins and are analogous to gonococcal Por. The class 4 and 5 proteins are analogous to gonococcal Rmp and Opa, respectively. Serogroup B and C meningococci have been further subdivided on the basis of serotype determinants located on the class 2 and 3 proteins. A handful of serotypes are associated with most cases of meningococcal disease, whereas other serotypes within the same serogroup rarely cause disease. All known group A strains have the same protein serotype antigens in the outer membrane. Another serotyping system exists based on the antigenic diversity of meningococcal LOS.

**Meningitis**

The term *meningitis* refers to inflammation the meninges of the brain or spinal cord. Meninges are any of the three membranes that envelope the brain and spinal cord. The disease *meningitis* is caused by a number of different bacteria and viruses. Bacterial causes include *Haemophilus influenzae*, *Escherichia coli*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Neisseria meningitidis*. Although a variety of cocci cause meningitis, the term *meningococcus* is reserved for the Gram-negative, bean-shaped diplococcus, *Neisseria meningitidis*. Like its relative *N. gonorrhoeae*, the organism tends to occur intracellularly in the cytoplasm of neutrophils which are attracted to the site of inflammation in the meninges, so this type of infection is called pyogenic (pus-forming).

Marchiafava and Celli were the first to report observing Gram-negative diplococci in cerebrospinal fluid of a fatal case of meningitis in 1884. In 1887, Weichselbaum isolated the bacterium from six cases of meningitis and established the isolates as a distinct species and proven to be the cause of meningitis.

**Pathogenesis**

Infection with *N. meningitidis* has two presentations, meningococcemia, characterized by skin lesions, and acute bacterial meningitis. The fulminant form of disease (with or without meningitis) is characterized by multisystem involvement and high mortality.

Infection is by aspiration of infective bacteria, which attach to epithelial cells of the nasopharyngeal and oropharyngeal mucosa, cross the mucosal barrier, and enter the bloodstream. If not clear whether blood-borne bacteria may enter the central nervous system and cause meningitis.

The mildest form of disease is a transient bacteremic illness characterized by a fever and malaise; symptoms resolve spontaneously in 1 to 2 days. The most serious form is the fulminant form of disease complicated by meningitis. The manifestations of meningococcal meningitis are similar to acute bacterial meningitis caused by other bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *E. coli*. Chills, fever, malaise, and headache are the usual manifestations of infection. Signs of meningeal inflammation are also present.

**Clinical manifestations of *N. meningitidis* infection**

The onset of meningococcal meningitis may be abrupt or insidious. Infants with meningococcal meningitis rarely display signs of meningeal irritation. Irritability and refusal to take food are typical; vomiting occurs early in the disease and may lead to dehydration. Fever is typically absent in children younger than 2 months of age. Hypothermia is more common in neonates. As the disease progresses, apnea, seizures, disturbances in motor tone, and coma may develop.
In older children and adults, specific symptoms and signs are usually present, with fever and altered mental status the most consistent findings. Headache is an early, prominent complaint and is usually very severe. Nausea, vomiting, and photophobia are also common symptoms.

Neurologic signs are common; approximately one-third of patients have convulsions or coma when first seen by a physician. Signs of meningeal irritation such as spinal rigidity, hamstring spasms and exaggerated reflexes are common.

Petechiae (minute hemorrhagic spots in the skin) or purpura (hemorrhages into the skin) occurs from the first to the third day of illness in 30 to 60% of patients with meningococcal disease, with or without meningitis. The lesions may be more prominent in areas of the skin subjected to pressure, such as the axillary folds, the belt line, or the back.

Fulminant meningococcemia occurs in 5 to 15% of patients with meningococcal disease and has a high mortality rate. It begins abruptly with sudden high fever, chills, myalgias, weakness, nausea, vomiting, and headache. Apprehension, restlessness, and delirium occur within the next few hours. Widespread purpuric and ecchymotic skin lesions appear suddenly. Typically, no signs of meningitis are present. Pulmonary insufficiency develops within a few hours, and many patients die within 24 hours of being hospitalized despite appropriate antibiotic therapy and intensive care.

**Figure 4. The characteristic skin rash (purpura) of meningococcal septicemia, caused by *Neisseria meningitidis***

**Virulence Factors**

For a time, the virulence of *Neisseria meningitidis* was attributed to the production of an "exotoxin" that could be recovered from culture filtrates of the organism. But when studies revealed that antitoxin reacted equally well with washed cells as culture filtrate, it was realized that the bacteria underwent autolysis during growth and released parts of their cell walls in a soluble form. Hence, the major toxin of *N. meningitidis* is its lipooligosaccharide, LOS, and its mechanism is endotoxic. The other important determinant of virulence of *N. meningitidis* is its antiphagocytic polysaccharide capsule.

The human nasopharynx is the only known reservoir of *N. meningitidis*. Meningococci are spread via respiratory droplets, and transmission requires aspiration of infective particles. Meningococci attach to the nonciliated columnar epithelial cells of the nasopharynx. Attachment is mediated by fimbriae and possibly by other outer membrane components. Invasion of the mucosal cells occurs by a mechanism similar to that observed with gonococci. Events involved after bloodstream invasion are unclear and how the meningococcus enters the central nervous system is not known.
Purified meningococcal LOS is highly toxic and is as lethal for mice as the LOS from *E. coli* or *Salmonella typhimurium*; however, meningococcal LOS is 5 to 10 times more effective than enteric LPS in eliciting a dermal Shwartzman phenomenon (a characteristic type of inflammatory reaction) in rabbits. Meningococcal LOS has been shown to suppress leukotriene B4 synthesis in human polymorphonuclear leukocytes. The loss of leukotriene B4 deprives the leukocytes of a strong chemokinetic and chemotactic factor.

**Host Defenses**

*N. meningitidis* establishes systemic infections only in individuals who lack serum bacterial antibodies directed against the capsular or noncapsular (cell wall) antigens of the invading strain, or in patients deficient in the late-acting complement components.

The integrity of the pharyngeal and respiratory epithelium appears to be important in protection from invasive disease. Chronic irritation of the mucosa due to dust or low humidity, or damage to the mucosa resulting from a concurrent upper respiratory infection, may be predisposing factors for invasive disease.

The presence of serum bactericidal IgG and IgM is probably the most important host factor in preventing invasive disease. These antibodies are directed against both capsular and noncapsular surface antigens. The antibodies are produced in response to colonization with carrier strains of *N. meningitidis*, as well as *N. lactamica*, and other nonpathogenic *Neisseria* species that are normal inhabitants of the upper respiratory tract. Protective antibodies are also stimulated by cross-reacting antigens on other bacterial species such as *Escherichia coli*. The role of bactericidal antibodies in prevention of invasive disease explains why high attack rates are seen in infants from 6 to 9 months old, the time at which maternal antibodies are being lost. Individuals with complement deficiencies (C5, C6, C7, or C8) may develop meningococcemia despite protective antibody. This emphasizes the importance of the complement system in defense against meningococcal disease.

**Epidemiology**

The meningococcus usually inhabits the human nasopharynx without causing detectable disease. This carrier state may last for a few days to months and is important because it not only provides a reservoir for meningococcal infection but also stimulates host immunity. Between 5 and 30% of normal individuals are carriers at any given time, yet few develop meningococcal disease. Carriage rates are highest in older children and young adults. Attack rates highest in infants 3 months to 1 year old. Meningococcal meningitis occurs both sporadically (mainly groups B and C meningococci) and in epidemics (mainly group A meningococci), with the highest incidence during late winter and early spring. Whenever group A strains become prevalent in the population, the incidence of meningitis increases markedly.

**Treatment**

Penicillin is the drug of choice to treat meningococcemia and meningococcal meningitis. Although penicillin does not penetrate the normal blood-brain barrier, it readily penetrates the blood-brain barrier when the meninges are acutely inflamed. Either chloramphenicol or a third-generation cephalosporin such as cefotaxime or ceftriaxone is used in persons allergic to penicillins.

Meningococcal disease is contracted through association with infected individuals, as evidenced by the 500- to 800-fold greater attack rate among household contacts than among the general population. Because such household members are at high risk, they require chemoprophylaxis. Sulfonamides were the chemoprophylactic agent of choice until the emergence of sulfonamide-resistant meningococci. At present, approximately 25 percent of clinical isolates of *N. meningitidis* in the United States are resistant to sulfonamides; nowadays, rifampin is the chemoprophylactic agent of choice.

**Control**

Groups A, C, AC, and ACYW135 capsular polysaccharide vaccines are available. However, the polysaccharide vaccines are ineffective in young children (in children under 1 year old, antibody levels
decline rapidly after immunization) and the duration of protection is limited in children vaccinated at 1 to 4 years of age. Routine vaccination is not currently recommended because the risk of infection is low. The group B capsular polysaccharide is a homopolymer of sialic acid and is not immunogenic in humans. A group B meningococcal vaccine consisting of outer membrane protein antigens has recently been developed, but is not licensed in the United States.

**Tailpiece**

**Search for a universal vaccine for meningococcal meningitis**

There is an obvious need for a universal vaccine for meningococcal meningitis, but the development of an effective vaccine against all forms of *N. meningitidis* has been hampered by the high degree of variation in the proteins on the surface of the bacterium which leads to the occurrence of many different antigenic types.

More than 10% of the population may be carrying the bacterium at any one time on the mucosal surfaces of the nose and throat. The majority of these carriers will not have any symptoms of the disease, but this continual exposure to the immune system puts pressure on the bacterium to mutate its surface components in order to survive. Thus, natural selection is the driving force for the emergence of new antigenic variants.

Among the class 2 and 3 outer membrane proteins of *N. meningitidis*, Por A has been considered a primary target for a vaccine-induced antibody. PorA is a major component of the outer membrane of *N. meningitidis*, and anti-PorA antibodies are thought to be a critical component in immunity. Interactions between antibodies and PorA have been studied. Different strains of the bacterium have different PorA amino acid sequences within the region of the protein that specifically binds to antibody molecules. PorA has several large amino acid "loop" regions that protrude from the surface, and it is these loops that are targets for antibody binding.

In the laboratory, the antigen-binding fragment (Fab) of anti-PorA antibodies can be crystallized and reacted with the antigenic loop regions of PorA in order to determine the specificity of binding between antigen and antibody. Slight changes in PorA amino acid sequence have been shown to cause loss in the ability to bind to antibody molecules. In nature, the bacterium mutates to insert new amino acid residues into the tip of the loop, which alters or eliminates many of the interactions with antibody and allows the bacterium to bypass previous immune responses.

![Image of the antibody (Fab) molecular surface, with the PorA antigen superimposed. The dark colored groove on the surface of the antibody matches precisely the shape of the PorA antigen; hence any changes in the sequence of PorA in this region can disrupt antibody binding. Jeremy Derrick, UMIST. SRS Annual Report.](image)

Hence, by introducing changes into portions of the PorA protein that are exposed at the surface, the bacterium can evade the attention of the immune system. These alterations are apparently introduced without compromising the biological function of PorA, as a pore-forming protein. Designing vaccines that are able to take into account these changes is a huge challenge, but as more information of this type becomes known, it leads to a more rational approach to design of a universal vaccine for meningococcal meningitis.