Pathogenetic of Neisseria meningitidis: a review of current understanding

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Abstract

Despite nearly 200 years of study and research we still do not know why Neisseria meningitidis causes severe disease with high morbidity and mortality, particularly epidemics lasting 5-10 years or longer. Much is now known about its transmission, the different clinical outcomes and their underlying pathophysiology and, in more recent years, using genetic techniques, new knowledge regarding the organism’s virulence factors. However it would seem that the pathogenesis of Neisseria meningitidis remains a complex interaction between the organism and its ability to change and develop various virulence factors such as PilC, Opc, LOS and capsular phase switching, and the host immune response.

Key words: Neisseria meningitidis, pathogenesis, host immune response, virulence factors

Introduction

Meningococcal meningitis was first described nearly 200 years ago, and since then, study and research has proved that there is a complex interrelationship between the organisms and the host. Meningococcal disease occurs worldwide, being endemic in temperate climates, with sporadic cases and small clusters in winter and spring as well as causing major epidemics. Over the last 20 years serogroup B meningococci have been responsible for significant epidemics in Europe, Latin America, New Zealand and more recently in the United States (1,2,3).

The clinical picture of acute meningococcal disease has been well defined, about 90% have meningitis either without septicaemia or with septicaemia and generalized macular or petechial skin lesions. About 10% of patients develop fulminating sepsis characterized by a brief period of high fever, overwhelming septicaemia with endotoxic shock, disseminated intravascular coagulation (DIC), little or no evidence of meningitis and numerous, extensive purpuric and ecchymotic lesions on the extremities (purpura fulminans). Mortality in these patients is about 44% (4,5).

Some patients present with only septicaemia, with no meningeval involvement. A recent study seems to suggest that increasing numbers of patients are presenting with just septicaemia (6).

However, despite all that is known in regard to transmission, disease outcome, host immunity, etc., we still do not know exactly why one person may develop invasive disease and others do not, or why epidemics occur with specific serotypes. A significant problem hampering the study of meningococcal disease has been the fact that Neisseria meningitidis is a uniquely human pathogen, thus the lack of suitable animal models that can fully reflect the complex microenvironment seen during the disease processes. A number of animals such as mice, monkeys, rabbits and chicken embryos have been used to gain some significant insights (7,8,9). Consequently, it has been mainly in vitro cell and organ cultures studies, using human cells, cell lines or tissues that have significantly contributed to the understanding of the pathogenic processes (10,11,12). With new molecular technologies now available we are able to gain further knowledge of the mechanisms involved.

Pathogenesis of invasive disease involves at least 4 stages, and in the case of meningitis 7 stages. These are 1. exposure to a pathogenic strain; 2. attachment to epithelial cells of the naso-oropharyngeal mucosa; 3. penetration of the mucosal barrier; 4. survival in the blood stream, then in meningitis; 5. entry into the cerebrospinal fluid (CSF); 6. survival in the CSF; and 7. disease production in the meninges and brain. These processes are influenced by bacterial properties, environmental conditions, preceding or concomitant infections as well as the immune status of the patient.

The aim of this review is to look at the current understanding of the pathogenesis of Neisseria meningitidis in regards to these stages, focussing on the bacterial properties and host mechanisms involved (Fig 1).

Stages of pathogenesis

Exposure to Neisseria meningitidis

The human naso-pharyngeal mucosa is the natural reservoir of Neisseria meningitidis, carriage rate being 5%-10% and up to 50% in crowded conditions where people are brought together from different regions e.g. military barracks and boarding schools. The carriage rate depends both on the rate of acquisition and duration of carriage. Serogroups A, B and C are responsible for most meningococcal cases. Although serogroup A can cause outbreaks, it has mainly been responsible for epidemics in sub-Saharan Africa. In developed countries serogroups B and C are responsible for most meningococcal disease, in particular serogroup B has been responsible for protracted epidemics in various parts of the world. New Zealand is now in its 12th year of an epidemic with phenotype B:4:P1.4 (13) (Refer Appendix 1).

Studies on meningococcal carriage have showed up to 50% of carrier strains were non-capsulated and until recently were considered non-pathogenic. It has now been shown that capsular expression is phase variable (14), and the loss of the capsule probably enhances the organism’s ability to colonize the nasopharynx (15). The carriage of particular strains did not prevent colonization with a heterologous strain (15). Vogel et al describe a case where the serogroup changed from serogroup C to serogroup B within days of transmission as a result of the transfer of serogroup-specific genes (16). Colonization occurs both on the exterior surface of the mucosal wall and intra- or sub-epithelially with damage to the nasopharyngeal ciliated epithelium possibly being the first step in colonization.

Physical damage by active or passive smoking (17), stressful events, prior upper respiratory tract infections due to viruses or Mycoplasma, and dry seasonal winds, e.g. in sub-Saharan Africa, are all predisposing risk factors for disease. Low socio-economic status is also a risk factor in that it is a marker for behavioural factors such poor nutritional status, hygiene and overcrowding.

Vaccines have been developed and used with some success to control and prevent infection however there are a number of problems with them (18). (Refer Appendix 2)

Attachment to epithelial cells of the naso-oropharyngeal mucosa

The various surface constituents of the bacteria such as the capsule, pili,
Figure 1 Overview - Pathogenesis of Neisseria meningitidis

- Transmissibility varies with serogroup
- Epithelial invasiveness varies with strain
- Strain variability
- External Factors: smoke, dryness, viral/mycoplasma infection

ATTACHMENT TO MUCOSA

Bacterial component
- Capsule - on-off regulation
- Pili
- OMP - Por A & Por B
  - Opa & Opc - inhibited by capsule
- Lipooligosaccharide
- Evasion of mucosal IgA - IgA proteases not absolutely required

- Large numbers confer specific site tropism
- Pili; class I & II
- Mediates endocytosis
- Pil C is main receptor on tip, binds to host epithelial & endothelial cells
- Phase variability

PENETRATION OF MUCOSA

Via Endocytosis
- Pili mediated
- Capsule & LOS sialylation
- Iron acquisition
- Opc binding to RGD protein
- Neisseria porins inhibit phagocytosis when translated to host cell membrane
- SOD inhibit phagocytosis

Wide range of cytokines
- Pro-inflammatory: TNF, IL-1
- Anti-inflammatory: IL-10, IL-12, TGF, IL-1ra
- Soluble cytokine receptors: LIF

- Vascular endothelium
- Purpura fulminans
- Coagulation involvement polymorphisms eg Leisden V & protein C deficiencies

SURVIVAL IN BLOODSTREAM

Capsule & LOS key
- Phase variability
- LOS sialylation important: inhibits complement activation
- Endotoxin release depends on strain
- Iron acquisition
- Host defences:
  - complement
  - MBL pathway
  - Antibodies
  - Neutrophils
  - Cytokines
- Meningococcal sepsis

- Blood-brain-barrier:
  - Transcytosis capillary endothelial cells
  - Between cells tight junction pil C adhere to meningeal cells

ENTRY TO CSF

Endotoxin induces local cytokines & chemokine production

Cytokine induced blood-brain permeability
- Neutrophil influx

SURVIVAL IN CSF

DISEASE PRODUCTION IN MENINGES AND BRAIN
class 1 porin, lipo oligosaccharide (LOS) and class 5 outer membrane proteins (Opa & Opc) are known to play a part in the invasion process, but it has been hard to define the exact process as the bacteria seem to display strong intra- and inter-strain variations. Virulence in the pathogenic neisseriae appears to be modulated, in part, in a stochastic manner. Random high-frequency biphasic on-off or antigenic switching involving multiple gene families seems to control the progression of bacteria through the host (12). Specificity of attachment of pathogens also depends on the host cell receptor sites (19).

**Capsule**

Capsular polysaccharide is an important and adaptive virulence determinant. As mentioned earlier, the capsule undergoes phase variation. Capsule-deficient meningococci may be more capable of invasion of the nasopharynx whereas capsulated strains survive immune detection and killing in the serum (20). Capsule expression is known to be controlled by genetic on-off switch mechanisms. It has recently been shown that meningococci have the ability to switch polysaccharide capsules through genetic transformation (14,20).

**Pili**

Pili appear to be an important surface component mediating initial attachment of meningococci to human epithelial and endothelial cells, and nasopharyngeal mucosa (21). McGee et al found that meningococci with pili attached to nasopharyngeal cells in greater numbers than those without pili, and the attachment of pilated meningococci differed among epithelial cells of different sites whereas non-piliated meningococci attached to all cell types in equal but low numbers (22).

Pili belong to the type IV family of pilin, similar to the pili of many other Gram-negative pathogens. They are thought to be glycoproteins that may be modified by glycans (23). Meningococci produce two types of pili, class I and class II, which are morphologically indistinguishable but functionally similar. They consist of at least two distinct sub units, PilE that forms the pilus fibre, and PilC that is found at the tip of the pili. PilC has been identified as the adhesin important for binding to epithelial and endothelial cells whereas PilE and other pili proteins influence the recognition of the PilC receptor (21,24). Nassif and So's work indicates that organisms expressing high-adhesive bundles of pili and PilC1 are more likely to colonize the host (24).

Piliated meningococci attach to the microvilli of non-ciliated epithelial cells and interact with them and undergo endocytosis by the cells (25,26). Pili are not only subject to phase variation but also to antigenic/structural variations. As well as intragenomic recombination, intergenomic recombinations are common because Neisseriae readily take up DNA from other lysed Neisseriae. (23)

**Outer membrane proteins (OMPs) - Class 1, 2 and 3 OMPs - PorA and PorB**

Porins are trimeric proteins that constitute pores that allow small solutes to diffuse through the outer membrane of the organisms. The neisserial porins are able to insert into the membranes of eukaryotic cells and have been shown in vitro to influence host neutrophil function thus inhibiting their phagocytosis. (27). They, like the other surface components, are subject to phase variation (28). This is particularly significant given PorA OMP is an important component of group B meningococcal vaccines.

**Class 5 OMP - Opa and Opc**

Opa and Opc protein play an important role in adherence as well as invasion in uncapsulated variants (24,29). A class of Opa proteins has been shown to bind to host receptors of the carcinoembryonic antigen (CEA) gene family (29) thus facilitating phagocytosis and cytokine production (30).

Opc significantly increases adhesion and invasion. Different isolates vary in the amount of Opc protein produced, and the more Opc produced, the greater the adhesion of the strain (24). Binding to host receptors stimulates engulfment of the bacteria by host epithelial cells (30). While in encapsulated bacteria, Opa and Opc do not affect bacterial interaction with host cells (24).

**LOS - endotoxin**

LOS of meningococci is an amphipathic molecule consisting of a hydrophilic carbohydrate portion and a hydrophobic lipid A portion that attaches to the outer membrane. It is structurally different from that of enteric gram-negative bacteria (31). The oligosaccharide portion of LOS mimics the glycosphingolipid antigens found on the surfaces of human cells including erythrocytes (32). Structural analysis has shown that this oligosaccharide not only varies serotypically between strains, but that there is considerable microheterogeneity within a single strain (32). The significance of this has yet to be determined.

The absence of LOS seems to inhibit attachment to epithelial cells and therefore invasion of the host cells (31). A recent study using a mouse model showed a meningococcus mutant defective in LOS sialylation did not colonize (7).

LOS also is subject to antigenic variation and can be modified by sialylation thus inhibiting Opa and Opc mediated interactions with the host cells (24).

To effectively attach and invade, the bacteria must also evade the mucosal immunoglobulin A (IgA), actively secreted by the plasma cells. This is achieved by the production of IgA proteases (33) that cleave the proline hinge region of IgA rendering it non-functional, allowing the bacterium to attach to the epithelium (34,35). Mulks et al showed strains of meningococci may produce two distinct IgA proteases and that each isolate elaborates either one or the other but not both (36). These IgA proteases have also been shown to be potent inducers of tumour necrosis factor -α (TNF-α) in peripheral mononuclear cells (37). Recently type 2 IgA1 protease was found to reduce lysosomal protein levels in the host epithelial cell, thus promoting intracellular survival of the bacteria (38). While these proteases contribute significantly to virulence they are not a requirement for an invasive strain (39).

A recent study showed meningococcal carriage elicited a mucosal immune response as well as a cellular one (40). Invasive disease occurs when the patient lacks serum antibody, thus it is significant to note that those most susceptible to meningococcal disease, i.e. those under 2 years old, will not yet have developed any specific antibodies.

**Penetration of the mucosal barrier**

Following mucosal adherence and a period of adaptation and proliferation,

Meningococci are thought to pass through the mucosal epithelium via phagocytic vacuoles as a result of endocytosis (30,22,24,25,26). During this invasion process, bacterial factors as discussed above, involved in attachment, modulate the metabolism of the mucosal cell including the host phagocytic system. However all the processes have not yet been fully elucidated. A study by De Vries et al using monolayers of primary cultured nasopharyngeal cells, suggests that a concurrent phase switching of multiple surface constituents may be required to establish an invasive bacterial phenotype (12).

While pili play a part in adherence to cells, it is unclear whether they actually promote bacterial cell entry. However, they do determine host and tissue tropism as well as other important pathogenic functions such as DNA uptake and bacterial movement (23).
Cell surface-located sialic acids of the capsule and LOS are both important in mediating resistance to phagocytosis and complement-mediated killing via alternative pathway activation (47). It has been shown that the possession of a (α 2→8)-linked polysialic acid capsule resulted in decreased bacterial binding to human macrophages, most probably by interfering with phagosome-lysosome maturation (42). Since macrophages are able to kill meningococci, this is an important virulence mechanism (42). The ability to switch between a non-sialylated and a sialylated phenotype enables the meningococci to adhere and invade the mucosal epithelial cells (41).

The ability to acquire iron from the host is essential for survival. Neisseriae produce outer membrane proteins - transferrin, lactoferrin and haem binding proteins that bind host transferrin, lactoferrin and haem and remove iron from them (24).

Opc binds arginine-glycine aspartate (RGD)-containing serum proteins such as vitronectin that leads to cellular invasion through the subsequent binding of the RGD protein to its cognate integrin receptors. Further investigation is required as to how these binding functions may help the bacteria to survive (29,43).

PorA and PorB have been shown to translocate spontaneously as functional ion channels into plasma membranes of the host cells where they inhibit neutrophil attachment and ingestion as well as cell signalling. (27). This and other functions observed in vitro require further study.

Meningococci also produce superoxide dismutases (SOD) that catalyze the conversion of superoxide into hydrogen peroxide and oxygen. Copper and zinc co-factor SOD (Cu, Zn SOD) is found in the periplasm and it is thought that they protect meningococci from uptake and killing by various phagocytic cells (44,45).

Survival in the blood stream

Meningococci can survive and proliferate in the blood stream because of particular bacterial virulence factors or incompleteness of the host defence system (30).

Bacterial capsulation and LOS play an essential role in the pathogenesis of meningococcal septicaemia (46,47). Klein et al showed that bacterial capsulation and LOS structure protected against complement-mediated bacteriolysis and phagocytosis (46). With dendritic cells (DC), it has been shown the capsule mainly inhibits adherence, thus inhibiting phagocytosis, where as sialylation of LOS prevents phagocytosis and subsequent phagocytic killing (47).

Meningococcal LOS sialylation confers significant serum resistance partly by inhibiting the alternative complement pathway activation and opsonization as well as masking the epitopes recognised by human serum (46,48) This is essential for their survival in blood. Sialylation decreases adherence of bacteria to endothelial cells, therefore LOS immunotypes that cannot be sialylated appear to predominate in nasal carriage strains, whereas immunotypes that can be sialylated tend to be found in the blood.

Endotoxins also have a direct action on the properdin pathway and activation of the classical pathway may be activated as a result from combination of endotoxin with natural antibodies present in the sera (49)

During growth and lysis of meningococci, endotoxin is released in the form of vesicular outer membrane structures consisting of up to 50% LOS and OMPs, lipids and capsular polysaccharides (30,50). Endotoxin is the main mediator of the proinflammatory response and cytokine induction (47). Strains isolated from patients with septic shock liberate more endotoxin than do those with benign meningococcaemia. However it is not just the level of LOS, but also the production of certain cytokines and their levels that correlate to clinical outcome. The significant cytokines seem to be proinflammatory TNF, interleukin-1β (IL-1β), IL-6, and anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra), IL-10, and transforming growth factor-beta (TGF-β) (57,52,53).

As mentioned earlier, the ability to acquire iron from the host is essential for survival. Meningococci have been shown to produce a haemoglobin receptor HmbR and a receptor HpuB for haemoglobin bound to haptoglobin, which are essential for the utilization of haemoglobin. Energy for the transport of iron from the host iron-binding proteins across the outer membrane into the periplasmic space is provided by outer membrane receptors tonB, exbB and exbB (54).

Host defences

Host defence after meningococcal invasion is determined by humoral and cellular responses belonging to the adaptive and innate immune systems. In meningococcal infection, the host inflammatory response is a major determinant of disease severity. The severity directly correlating with the degree of host inflammatory cell activation (46,55). The key components being complement, antibodies and neutrophil production (56,30).

Complement

The complement system plays a significant role in the host inflammatory response, being involved in a number of processes such as mediating chemostaxis of neutrophils, opsonization of organisms, inducing vasodilatation and enhancing vascular permeability (57).

Complement activation can occur through the classical pathway by antigen-antibody complexes, the alternate pathway by bacteria or LOS, and the mannose-binding lectin (MBL) pathway. As those who with meningococcal infection do not have antibodies, complement activation occurs by the alternate and MBL pathways.

The alternate pathway involves the interaction of factor B, factor D and properdin to generate C3b. Alternative pathway defects e.g. X-linked properdin deficiencies may predispose to overwhelming disease (58,59).

Inherited deficiencies in other complement components have been reported in association with severe or recurrent meningococcal disease (60,61,62,63). The significant components are those of the membrane-attack-complex (MAC). This complex is necessary for forming a lytic channel in Neisseriae as extra cellular lysis is a major mechanism in killing these organisms (64). It has been suggested that it is the insertion of the MAC into the bacteria and not the quantity formed on the surface that is important (65). However, due to the rarity of these deficiencies, they could only be only a small number of cases (30).

MBL pathway

MBL is a sugar-binding protein belonging to a family of calcium-dependent collagenous lectins, most of which are components of the innate immune system found in the serum of humans (49). On binding to its targets, MBL activates the complement system via MBL-associated serine proteases (MASP-1 and MASP-2. MASP-2 cleaves C4 and C2 to generate a C3 convertase independently of immunoglobulin (66,67). MBL may also interact directly with phagocytic cells to regulate their function. Serum levels of MBL are genetically determined via 3 structural gene mutations and 3 promoter region polymorphisms (68).

It has been reported that some genetically determined variants might predispose individuals to meningococcal infection (69,68,49,70). The expression of capsular polysaccharide has been shown to decrease the binding of MBL to N meningitidis serogroup B as does the structure and composition of bacterial endotoxins (67).

However it seems the lack of the sialic acid acceptor site or removal of sialic acid rather than encapsulation, which is important in
determining MBL binding to bacteria.

**Antibodies**

Studies have shown that susceptibility to systemic meningococcal disease is related to a deficiency of humoral antibodies to meningococci. There is an inverse correlation between serum bactericidal activity and age-related incidence of meningococcal infection with the risk of disease decreasing with increasing age. At least 40-50% of cases occur in children under 5 years, most under one year old (71,72,73). Antibodies can be acquired either by passive immunization by transplacental passage of immunoglobulins or active sensitization due to the meningococcal carrier state. Goldschneider et al's study suggested that natural immunity to meningococcal disease is initiated, reinforced, and broadened by intermittent carriage of different strains of meningococci throughout life (74). Epidemics tend to show a shift in the number of cases towards older age groups, in part due to them having no previous exposure to that particular strain (72,71). It is interesting to note that in the New Zealand epidemic, there has not been the shift to the older age groups, with most cases still occurring in those under 1% years of age - in 2004 44% were under 5 years (71).

The bacterial activity of normal serum to meningococci can be attributed largely to γM globulin. Hobbs et al reported low γM globulin in patients with fulminating meningococcal septicemia patients, suggesting these were most likely inherited deficiencies (75). IgG subclass deficiency has also been reported as being a predisposing factor for recurrent meningococcal disease (76). Circulating IgA antibody to *N meningitidis* capable of blocking the complement -mediated bactericidal activity of IgG and IgM may also make people more susceptible (77). However the role of blocking IgA antibody is still uncertain (30,73).

Cellular immunity defects may also predispose an individual to disease. Immunosuppressive drugs and autoimmune disease are reported predisposing factors. Splenectomy has also been well defined as a risk factor for overwhelming disease with encapsulated bacteria (30).

**Neutrophils**

Neutrophils are natural effector cells mediating antimicrobial defence by the production of a wide range of cytokines (78). Opsonic and non-opsonic phagocytosis by neutrophils, as well as complement-mediated bactericidal activity in serum, are important in the host defence against *Neisseria meningitidis* (56,30).

Neutrophils are both friend and foe. They are essential for the eradication of pathogens, but the oxidants and proteases they release as a result of activation and adhesion are also toxic to host tissue (79,46).

**Cytokines**

Endotoxins, as discussed earlier, induce the production of a wide range of cytokine. Pro-inflammatory cytokines, particularly TNF and IL-1 are essential for adequate host defence (52,53,80,81). Their functions include inducing chemokines and adhesion molecules as well as stimulating phagocytosis and tissue repair. In high concentrations, TNF-α can cause wasting of muscle, septic shock and death (53). The pro-inflammatory effects of IL-1 are mediated by IL-1α that is mainly cell-bound and IL-1β, which is released. IL-1β is considered an important mediator in the complex pathogenesis of septic shock and bacterial meningitis (81).

Likewise anti-inflammatory cytokines and chemokines, eg IL-10, IL-12, TGF-β, and IL-1ra are also produced (52,53). These anti-inflammatory cytokines inhibit the host immune response. The anti-inflammatory response also includes soluble cytokine receptors which down regulate their specific cytokine e.g. TNF activity is down regulated by soluble TNF receptor (sTNFR) (80). Principle source for cytokines are blood monocytes, tissue macrophages (52) as well as specialised antigen-presenting cells such as dendritic cells (82,47).

Another recently described cytokine, cytokine leukemia inhibitory factor (LI), can be produced by a number of cells, including fibroblasts, monocytes, macrophages, T lymphocytes, and endothelial cells (83). It stimulates the acute-phase response, primes the immune system and phagocytic cells. LI synthesis can be induced by cytokines TNF, IL-1 and endotoxin. Along with other acute-phase cytokines it contributes to pathogenesis of septic shock (83).

**Meningococcal sepsis**

Sepsis occurs when the immune system is severely compromised and unable to eradicate pathogens (79). Septic shock results in marked inflammatory response, leading to capillary leakage, DIC, vascular injury, and, ultimately death. Complement plays a key role in that it contributes to the host's defence but may also contribute to tissue damage and severe complications (57).

In the early stage of disease both pro and anti-inflammatory cytokines are increased, probably as a result of generalized activation (84). However, in severe shock, although both pro and anti-inflammatory cytokine levels are raised, the levels of anti-inflammatory are much higher (85). van Dissel et al found, by measuring TNF-α and IL-10 levels in febrile patients, that the ratio of IL-10 (anti-inflammatory) to TNF-α nearly doubled in those patients who died.

A major target for inflammatory response in meningococcal disease is the vascular endothelium. Recent studies have shown that meningococci have the capacity to bind endothelial cells in a receptor-ligand-specific fashion and that this bacterium-endothelium contact may be critical in mediating the vascular damage seen in this disease (86). Dixon et al demonstrated that LOS was important in determining the pattern of vascular endothelial adhesion molecule expression and that even subtle changes in the LOS structure influenced the host inflammatory response (86).

Purpura fulminans is a syndrome characterised by intravascular thrombosis and haemorrhagic infarction of skin, limbs and digits. The clinical presentation of skin haemorrhages is characterized by endothelial damage with haemorrhage around and microthrombi within the small vessels (87). The lesions are a reflection of the endotoxin and cytokine vasculitits mediated by the up regulation of adhesion molecules on endothelium and degranulating activated neutrophils.

Recently Harrison et al demonstrated that meningococci that reached the skin invade the deep tissues, forming micro colonies that expressed three key virulence factors - capsule, PorA, and pilin (88) with phase variation occurring. These findings confirm in vitro studies regarding their importance in pathogenesis of meningococcal disease. Fullmant meningococcal sepsis also involves the coagulation systems, which is different to meningitis where coagulation factors are not involved. It has been shown that deficiency of Protein C, a regulator involved in the coagulation, anticoagulation and fibrinolytic systems, leads to extensive DIC and necrosis (89,90). Recent work by Faust et al has shown that the endothelial protein C activation pathways are impaired (91).

The Factor V Leiden mutation that has been associated with thrombosis may be a risk factor for developing fulminant meningococcal disease. However, it is most likely a combination of factors are responsible for the dysregulation of the coagulation cascade in meningococcal disease, including low levels of antithrombin III, protein C and Protein S, up-regulation of tissue factor expression on monocytes, and high plasminogen activator inhibitor 1 (92,93).
Entry into the cerebrospinal fluid

Compared to other extracellular pathogens, meningococci have a tendency to invade the meninges. From the blood, meningococci can gain access to the meninges by crossing the blood-brain-barrier (BBB). Entry is gained either by transcytosis through capillary endothelial cells or by passage between the cells after disruption of the tight junction (24). Alternatively, the choroidal plexuses, the major site of CSF synthesis, is another possible route into the CSF. The choroidal plexuses are situated in the ventricles of the brain as leaf-like structures with a central core of blood vessels covered either side by epithelium. To cross the blood-brain barrier at this site would involve crossing the epithelial layer (24). Dural trauma has also been implicated as a risk factor for meningitis (94).

Neisseria meningitidis shows a specific predilection for binding to the leptomeninges and meningeal blood vessels in human brain but not to the cerebral cortex (95). While the mechanisms by which meningococci gain access to the subarachnoid space and the molecular bases of the interactions between the meningococci and cells of the leptomeninges are poorly understood, in vitro studies showed the major ligand that mediated adherence of meningococci to both meningo cells and leptomeninges appeared to be the pili (95). Pron et al showed PIlC played a significant role in the crossing of the BBB, most likely through pilus-mediated adhesion (96).

Survival in the CSF

Having crossed the blood-brain barrier and into the subarachnoid space, where the main humoral and cellular host defences are absent, meningococci can proliferate uncontrolled (30). Endotoxins induces proinflammatory cytokines, e.g. TNF, IL-1, IL-6, platelet activating factor, and anti-inflammatory cytokines, e.g. IL-1Ra, IL-10 (95). Increased levels of chemokine IL-8 (CXCL8), growth-related oncogene alpha (CXCL11), monocyte chemotactic protein 1 (MCP-1), and macrophage inflammatory protein 1a (MIP-1α) and (MIP-1β) are also seen (97). Cytokine production precedes to the rapid influx of neutrophils and later, monocytes and T cells (53,9).

Wells et al’s study, using human meningo cellular cells, showed that these cytokines and chemokines were most probably from the cells of the meninges (97). These cytokines are produced by the cells of the meninges and their function is confined to the local inflammatory reaction in the CSF compartment. (97,9).

Disease production in the meninges and brain

Studies have shown that there are separate compartmentalised intravascular & intracranial inflammatory responses to infection (82,9). Cytokines IL-1 and TNF enhance the permeability of the blood-brain barrier and promote the influx of neutrophils by up regulation of adherence molecules. The subsequent release of neutrophil products contributes to the development of clinically overt meningitis (30). Chemo attractant and activator, N-formyl-L-methionyl-L-leucyl-L-phenylalamine (fMLP) that is produced during bacterial cell lysis also contributes to the infiltration of granulocytes (53).

The anti inflammatory cytokines IL-10 and transforming growth factor-β (TGF-β) which control the pro-inflammatory cytokines may in certain situations impair host defences (53).

Other bacterial considerations

As discussed earlier, the bacterial constituents important in pathogenesis show strong intra- and inter-strain variations. These phase variations are associated with reversible mutations at individual loci termed “contingency loci” (98,99). Loci have been identified associated with various function-related gene groups such as evasins, LOS biosynthesis, adhesins, iron acquisition and restriction-modification systems (98). Many mobile DNA elements transpose from one chromosomal location to another (100). They include insertion sequences, transposons, phages and pathogenicity islands (100,101). In pathogenic bacteria it is thought that some of these elements may be responsible for the efflux of genetic material coding for virulence traits (100). For example, Kahler et al described an exchangeable DNA island ex that consists of at least 3 distinct cassettes, one of which encodes the Ib receptor (101).

RTX-cytotoxin- related proteins, two Fe-regulated proteins FrpA and FrpC, have recently been described associated with and secreted by the outer membrane (102,103,104). Although the functions of these meningococcal RTX-like proteins are unknown, given they are related to other bacterial cytotoxins it is possible they may contribute to the severity of disease (104,105). In a recent study they did not appear to have any toxic affect on infant mice, however, other roles in disease process could not be excluded (106).

Dendritic cells are specialized antigen-presenting cells found as a trace population in most tissues, when activated, start to capture and process antigens. Dendritic cells appear to play a crucial role both in the initiation and modulation of specific immune responses as well determining the type of response (82,107). Further study into dendritic cell-bacterial interactions may be useful in finding ways to control the disease severity.

Bacteria have mechanisms known as quorum sensing (108) that co-ordinate gene expression in response to population density (109). These involve the production and detection of signalling molecules (auto-inducers), which modulate critical functions including virulence factor production. Winzer et al describe the presence of a luxS gene that is required for the production of a signal molecule called autoinducer-2 (AII) (109). Their study showed this gene contributed to the virulence of the organism.

Conclusions

Because meningococci only interact with human cells, our knowledge of their colonization and pathogenesis has been derived largely from sub-optimal study models.

However, studies have shown that meningococci have greater genetic diversity than most other human bacterial pathogens (30) and display strong intra and inter strain variation in their surface constituents (12,110). Pathogenic meningococci are able to regulate their virulence at many levels (22) and it has been shown that bacterial cell interactions are essential not only for pathogenesis, but also for other aspects of their survival and dissemination (111,99,112,113).

The capsule, LOS, PILC1, Opc and certain Opa are important bacterial components in the initial adhesion and invasion, and then once in the cells other virulence factors also play a part in survival and induction of the disease process (24). Highly significant in pathogenesis is the degree to which pathogenic meningococci regulate their virulence factors throughout the different stages of the disease process (98). Expression of these factors are regulated either by phase/antigenic switching, e.g. pil and LOS, or environmental stimuli e.g. they undergo a variety of genetic modifications of their outer membrane proteins, particularly in respect to utilising host transferrin in an iron-depleted environment (101,98,100).

Using molecular typing methods it seems that invasive disease and significant epidemics outbreaks are caused by a few pathogenic meningococcal clones spreading around the world (30,15). It is still largely unclear why only some regions are affected, and compared with the prevalence of meningococcal carriage and transmission, invasive disease is relatively uncommon. However, it is known that certain host...
and environmental factors do increase the risk of invasive disease e.g. prior upper respiratory tract infection or overcrowded conditions.

The host immune response to meningococci also plays an important role in determining disease occurrence and severity. As van Deuren et al noted, invasive disease seems to occur only in patients lacking specific bactericidal or opsonizing antibodies (30). Carriage of meningococci may elicit an antibody response for a specific strain itself and which can cross-react with heterologous strains (40). However, colonization does not protect against re-colonization with that same strain, heterologous strains, or always protect against invasive disease (40). In contrast, the immune response elicited by invasive disease does cross protect against recurrence of disease (74). Since Neisseria meningitidis continues to be the major cause of bacterial meningitis and septicaemia in children, with high associated morbidity and mortality, it is important to develop effective vaccines to prevent meningococcal disease.

It is clear that both bacterial and host factors play important roles in pathogenesis, however there still remains many unanswered questions with regards to all the complex interrelationship processes involved. It has been suggested that certain genetic genes are required for bacterial transmission and pathogenesis (113).

Little is yet known about the regulatory networks responsible for the expression of Neisseria meningitidis virulence factors. With the now available technology for genetic analysis, there has been increased knowledge regarding the complexities involved in meningococcal disease. For example, Dietrich et al found that when bacteria adhered to human epithelial cells, 72 genes ORFs were differentially regulated and to brain endothelial cells 48 ORFs (112).

It has been shown that genetic factors influence an individual’s susceptibility to meningococcal disease (79). Genetic alterations that have been identified include polymorphisms in TNF receptors, IL-1 receptors, Fcy receptors and TLRs, all of which influence cytokine production and therefore the inflammatory response (114,115,79).

Further understanding of the processes involved in pathogenesis, particularly the regulatory pathways is important for future strategies in the prevention of meningococcal disease.

Appendix 1
Epidemiology of meningococcal disease

Meningococci are divided into 13 different serogroups using their capsular polysaccharides: A, B, C, D, W-135, X, Y, Z, Z’ (usually referred to as 29E), H, I, K, and L (116). There appears to be a difference in the transmissibility of the different serogroups with most disease world-wide caused by five serogroups, A, B, C, W-135, and Y, in particular serogroups A, B and C. Serogroups A and C, which tend to cause outbreaks in closed communities, are easily transmitted whereas certain epidemic strains of serogroup B have low transmissibility but high virulence (117). Serogroup A and C epidemics usually resolve in 1 to 3 years however serogroup B outbreaks begin slowly and may persist for decades, with a significant number of the cases occurring in children under 5 years old (118). Since 1974 there have been significant serogroup B epidemics in Norway, England, Latin America and more recently in the 1990's in New Zealand, Belgium and the United States (1,13).

There are currently 20 serotypes defined based on Class 2 and 3 Outer membrane proteins (OMP) and at least 10 types based on Class 1 OMPS (116). Thus B:4:P1.7b.4 (the current NZ epidemic strain) represents serogroup B, serotype 4 and serosubtype P1.7b.4 (119).

It is also important to note that most cases of meningococcal disease are caused by a small number of genetically defined clonal groups, e.g. in the last 20 years, electrophoretic type (ET) -5 has been the cause of the outbreaks in northern Europe and Latin America as well as New Zealand and the US Pacific Northwest (1,13,124), they do show significant strain variations. Results from the study by Tappero et al showed vaccines made from the class 1 OMPS from the epidemic strain are more effective than those from other strains in protecting that particular population (3).

New Zealand has developed a strain B:4:P1.7b.4 PorA specific vesicle vaccine that is currently being trialled (125).

A number of other non capsular meningococcal antigens have also been studied for possible inclusion in vaccines: transferrin binding proteins TbpA and TbpB can elicit a strong immune response and could be useful being included in a vaccine (126,127), neisserial surface protein A (NspA) (128,129), as well as Opa and Opv.

The ability of N meningitidis to “switch capsules” by genetic transformation introduces another challenge to find a suitable vaccine (14).

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