

Chapter 2

Classification and Pathogenesis of Meningococcal Infections

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Abstract

The clinical symptoms induced by *Neisseria meningitidis* reflect compartmentalized intravascular and intracranial bacterial growth and inflammation. In this chapter, we describe a classification system for meningococcal disease based on the nature of the clinical symptoms. Meningococci invade the subarachnoid space and cause meningitis in as many as 50–70% of patients. The bacteremic phase is moderate in patients with meningitis and mild systemic meningococcemia but graded high in patients with septic shock. Three landmark studies using this classification system and comprising 862 patients showed that 37–49% developed meningitis without shock, 10–18% shock without meningitis, 7–12% shock and meningitis, and 18–33% had mild meningococcemia without shock or meningitis. *N. meningitidis* lipopolysaccharide (LPS) is the principal trigger of the innate immune system via activation of the Toll-like receptor 4-MD2 cell surface receptor complex on myeloid and nonmyeloid human cells. The intracellular signals are conveyed via MyD88-dependent and -independent pathways altering the expression of >4,600 genes in target cells such as monocytes. However, non-LPS molecules contribute to inflammation, but 10–100-fold higher concentrations are required to reach the same responses as induced by LPS. Activation of the complement and coagulation systems is related to the bacterial load in the circulation and contributes to the development of shock, organ dysfunction, thrombus formation, bleeding, and long-term complications in patients. Despite rapid intervention and advances in patient intensive care, why as many as 30% of patients with systemic meningococcal disease develop massive meningococcemia leading to shock and death is still not understood.

Key words: Meningococcal meningitis, Septicemia, Classification, Lipopolysaccharide, Shock

1. Introduction

Neisseria meningitidis is an obligate human Gram-negative diplococcus residing asymptotically in the upper respiratory tract (1). The highest carriage rate is found among adolescents and young adults representing the main reservoir of the bacterium. Few young children carry meningococci (1). Most carried strains never cause

invasive disease. A limited number of meningococci belonging to specific clones or clonal complexes with serogroups A, B, C, Y, and W135 cause >95% of systemic meningococcal disease (SMD) (1). Encapsulated meningococci expressing pili, opacity proteins, and other subcapsular adhesion molecules are transferred by droplets or by direct contact (kissing) from an asymptomatic carrier to a nonimmune person.

After attachment to nonciliated columnar epithelial cells in the nasopharynx or the epithelium covering the tonsils, meningococci adapt and start to proliferate. Type 4 pili and various outer membrane proteins including opacity proteins undergo phase variation (2). Studies in vitro suggest that *N. meningitidis* may transverse the epithelial cells through “parasite directed endocytosis” (3). It is assumed that meningococci enter the blood stream through the capillaries and small veins in the underlying submucosal tissue. Survival and growth of encapsulated *N. meningitidis* in the circulation is a fundamental requirement for developing SMD.

Clinical disease usually develops within a week following breach of the mucosal barrier. A century ago, before any effective treatment existed, 70–90% of the patients died during the natural course of infection (4). After the introduction of antimeningococcal serum therapy in 1905, the case fatality rate (CFR) declined to approximately 40% (4). Treatment with sulfonamides in 1937 and with penicillin in 1945 resulted in a further decline of the CFR to approximately 10%. In the last decade, the CFR has ranged from 7 to 11% in Europe and the USA for sporadic cases, increasing to above 20% in outbreak situations (5–7).

Two characteristics make *N. meningitidis* unique among human pathogens: (1) The propensity to invade the meninges and (2) the ability to proliferate rapidly in the blood leading to shock and multiple organ failure in as many as 30% of the patients contracting serogroup B and C strains (6, 8, 9). Meningococci are known among physicians, epidemiologists, and lay people as the bacterium causing outbreaks of meningitis. In industrialized countries, physicians should rightly fear *N. meningitidis* as the bacterium that may cause overwhelming septicemia, shock, and death. Meningococci may kill previously healthy children or adults within 12–24 h (6, 10, 11). Septic shock and multiple organ failure is the direct cause of death in nine out of ten patients with lethal meningococcal infections in Western countries (Table 1) (6, 8–11). A minority dies of meningitis leading to brain edema, herniation, and arrest of brain circulation (6, 9–11). In Third World countries, particularly in sub-Saharan Africa, *N. meningitidis* serogroup A still causes large-scale outbreaks involving tens of thousands of people. Meningitis without shock appears to be the dominant clinical presentation (12).

A lack of bactericidal antibodies is the single most important predisposing factor for developing SMD (11, 13, 14). Defects in

Table 1
Classification of systemic meningococcal disease presentations

References	Year	<i>n</i>	Shock without meningitis (%)	Shock + meningitis (%)	CFR (%)	Shock without meningitis (%)	CFR (%)	Meningitis without shock (%)	CFR (%)	Mild meningococemia (%)	CFR (%)
(8)	1983	115	18	15	52	12	49	18	0	18	0
(9)	1987	206	17	13	29	7	37	33	1	33	0
(6)	2008	541	10	20	16	11	49	21	1	21	5

n denotes the total number of patients included in the study. CFR denotes case fatality rate of the previous column (6, 8, 9). The clinical presentations are defined as follows: Shock without meningitis: (1) severe septic shock lasting for 24 h or until death requiring fluid and pressor drug therapy and (2) $<100 \times 10^6/L$ leukocytes in CSF. Spinal puncture is presently not recommended for these shock patients. The leukocyte count is therefore substituted by clinical signs of distinct meningism. Meningitis without shock: (1) clinical distinct symptoms and signs of meningism, $\geq 100 \times 10^6/L$ leukocytes in CSF (or if the results of CSF are not obtainable, signs of distinct meningism) and (2) lack of persistent septic shock lasting >24 h requiring fluid and pressor drug therapy. Shock and meningitis: (1) severe septic shock lasting for 24 h or until death requiring fluid and pressor drug therapy and (2) $\geq 100 \times 10^6/L$ leukocytes (or if the results of CSF are not obtainable, signs of distinct meningism). Meningococemia without shock or meningitis (mild SMD): Meningococemia detected by blood culture, PCR or by other methods without (1) severe septic shock requiring treatment for at least 24 h and (2) $<100 \times 10^6/L$ leukocytes (or if the results of CSF are not obtainable, lack of marked meningism).

the alternative and terminal pathways of complement increase the risk of contracting invasive infection (11, 13, 14). Cohort and genome-wide association studies suggest that complement factor H and related proteins determine the susceptibility to meningococemia, whereas the role of mannose binding lectin is uncertain as a predisposing factor (15, 16). Meta-analysis suggests that genes related to fibrinolysis and interleukin-1 influence the severity of outcome (17).

2. Clinical Classification of Systemic Meningococcal Disease for Research Purposes

In the 1960s, it was recognized that patients with meningococcal infections presenting with the classical symptoms of meningitis (nuchal and back rigidity) and marked pleocytosis, i.e., $\geq 100 \times 10^6/L$ leukocytes in the cerebrospinal fluid (CSF) had a much better prognosis than patients with shock and minimal pleocytosis, i.e., $< 100 \times 10^6/L$ leukocytes in the CSF (18). Development of shock was the decisive factor determining survival or death and this conclusion was later confirmed by others (6, 8–11, 14, 19, 20). The degree of pleocytosis, i.e., the number of leukocytes in CSF $\geq 100 \times 10^6/L$ leukocytes became a cut-off marker for defining distinct meningitis in meningococcal research. Based on two easy recognizable criteria: (1) shock and (2) meningitis ($\geq 100 \times 10^6/L$ leukocytes in CSF or distinct signs of meningitis if CSF is not available) a classification system was established for research purposes (8). By applying this classification system in subsequent clinical studies it was possible to delineate the inflammatory responses induced by *N. meningitidis* and to describe the underlying pathophysiology in great detail (11, 14, 19, 20).

Three landmark studies used this clinical classification and comprised 862 patients, of whom 656 were studied prospectively, infected with serogroup B or C strains (6, 8, 9) (Table 1). Seventy percent of the 862 patients did not develop septic shock. They presented with distinct meningitis or mild meningococemia. The CFR was low (Table 1). Thirty percent of the patients developed septic shock with a much higher CFR than the nonshock group (Table 1). The majority of the shock patients lacked distinct signs of meningitis. Persistent shock was the major cause of death (Table 1).

2.1. Shock Without Meningitis (Fulminant Septicemia, Waterhouse–Friderichsen Syndrome)

The symptoms develop rapidly. The median time between the onset of symptoms to hospital admission was 12–13 h in five Western European studies (6, 11, 19). The clinical picture is dominated by septic shock requiring large volumes of fluids and vasoactive drugs, artificial ventilation due to acute respiratory distress syndrome (ARDS), renal failure, large hemorrhagic skin lesions, and adrenal hemorrhage reflecting severe disseminated intravascular

Table 2

Plasma levels of *Neisseria meningitidis* DNA copy numbers and LPS levels in 65 Norwegian patients with confirmed SMD

	Shock (<i>n</i>=21)	Shock + meningitis (<i>n</i>=2)	Meningitis without shock (<i>n</i>=28)	Mild meningococemia (<i>n</i>=14)
Nm DNA copies	2×10^7	1×10^6	$<10^3$	7.7×10^3
LPS EU/mL	43	9.4	<0.5	<0.5

n denotes the number of patients analyzed. All levels are given as median values (23). SMD denotes systemic meningococcal disease

Table 3

Plasma LPS levels vs. clinical presentation in 150 Norwegian patients with confirmed SMD

LPS (EU/mL)	Number of patients	Number dead	Case fatality rate (%)
>250	7	7	100
250–50	20	17	85
50–10	24	6	25
10–0.5	31	1	3
<0.5	68	0	0

EU/mL denotes endotoxin units per mL as determined by the limulus amebocyte lysate (LAL) assay. SMD denotes systemic meningococcal disease. The *dashed line* indicates the plasma shock level (10 EU/mL) (10, 19)

coagulation (DIC) (11, 14, 19). Patients lack marked symptoms and signs of meningitis, reveal minimal pleocytosis, and few meningococci are present in the CSF. This clinical presentation occurred in 10–18% of the 862 patients studied. The CFR varied from 16 to 52% depending on the pathogenicity of the strain and between the endemic vs. epidemic situation (5, 6, 8–11, 14, 19, 20). The severity, development of shock and death are closely associated with the level of meningococci in the circulation as measured by the number of *N. meningitidis* DNA copies reaching levels as high as 10^8 /mL (21–23) (Table 2). The plasma level of lipopolysaccharide (LPS, endotoxin) as quantified by the Limulus Amebocyte Lysate (LAL) assay is as high as 2,150 Endotoxin Units (EU)/mL (23). If the LPS level in plasma passes 8–10 EU/mL, 95% of the patients will develop septic shock (10, 19) (Tables 2 and 3).

The exponentially escalating numbers of meningococci induce an exaggerated and destructive inflammatory response in the

vasculature, heart, kidneys, and lungs with high levels of pro- and anti-inflammatory cytokines (11, 19). Fifty percent of nonsurviving patients die within 12 h after hospital admission (6, 10, 11, 19). Surviving patients may require treatment for weeks with prolonged ventilation for ARDS, dialysis for renal failure, amputation of peripheral part of the extremities, and extensive skin grafting. The late complications are a consequence of DIC leading to thrombosis in various organs. The CFR varied from 16 to 52% in the three studies comprising these 862 patients (Table 1).

Petechial rash, i.e., small hemorrhagic skin lesions ($\varnothing = 1\text{--}4\text{ mm}$) is common and one of the most characteristic symptoms of SMD. The rash combined with fever is considered the hallmark of this infection. Petechiae are, however, not universally present. Depending on meningococcal strains and host factors, typical hemorrhagic lesions are observed in 28–78% of the patients (6, 14). The petechial lesion is a consequence of a preceding meningococcemia, local attachment of meningococci to the endothelial cells of capillaries and small veins in the skin. The meningococci express capsule polysaccharide, type IV pili, and PorA outer membrane protein (24). The organisms “dock” at and migrate through, or between, endothelial cells, altering the antithrombotic surface of the endothelium. The result is formation of thrombi and extravasation of erythrocytes, which is seen as skin hemorrhages (11, 14, 19). The meningococci may divide locally and can be cultured 13 h after initiation of antibiotic treatment (25). Up to 100 meningococci have been visualized in a small area in biopsies of skin lesions (24). Hemorrhagic lesions with a diameter $>1\text{ cm}$ (ecchymoses) are primarily observed in patients developing shock with high levels ($>10\text{ EU/mL}$) of LPS. The presence of these lesions indicates severe DIC (11, 14, 19).

The hemorrhagic skin lesions and thrombosis in different organs are associated with massive up-regulation of tissue factor (TF) in circulating monocytes (11, 14, 19). TF activates the extrinsic pathway of the coagulation system. Concomitantly, the fibrinolysis is initially activated by tissue plasminogen activator (tPA). Subsequently, tPA is blocked by plasminogen activator inhibitor-1 (PAI-1) facilitating thrombi formation. Plasma fibrinogen is consumed with declining plasma levels (11, 14, 19). Activation of thrombin and plasmin is reflected in increased levels of thrombin–antithrombin (TAT) and plasmin–antiplasmin (PAP) complexes in plasma. The natural coagulation inhibitor protein C is reduced to 10–20% of normal levels (19). Reduction to such low levels is associated with purpura fulminans, i.e., diffuse thromboses of skin vessels (19). Antithrombin is often reduced to 50% of normal levels (11, 14, 19). The functional levels of tissue factor pathway inhibitor (TFPI) increases (19). The platelets are activated, adhere to surface structures on the vessel walls, and contribute to the formation of thrombi. The coagulopathy, which is more pronounced

than in most other cases of severe sepsis, is a consequence of the very high number of meningococci and the accompanying endotoxemia in the blood.

Shock, i.e., persistent hypotension and hypoperfusion of different organs, is the single most important indicator of survival or death in SMD (6, 8–11, 14, 18–20, 26). Persistent shock after fluid resuscitation is a consequence of vasodilation, vascular leakage, and gradually reduced contractility of the myocardium (11, 14, 19, 26). After volume treatment, the circulation is initially hyperdynamic with increased cardiac output and tachycardia compensating for low peripheral resistance. The myocardial contractility is gradually reduced, which is not compensated by massive fluid treatment and vasopressors (dopamine, norepinephrine, epinephrine, and dobutamine). The declining myocardial performance can be visualized by serially ultrasonographic examinations. At the terminal shock stage, the myocardial contractility and ejection fraction are severely reduced and the peripheral vascular resistance low. The patients often die of arrhythmia after a period of tissue hypoxia and acidosis (10). A myriad of factors contribute to the reduced cardiovascular performance, including nitric oxide (NO) produced locally in the endothelium and myocytes after up-regulating nitric oxide synthase 2 (NOS 2) (27).

2.2. Shock and Meningitis

The patients present with shock in combination with a marked pleocytosis or clinical symptoms of meningitis. This combination occurred in 13–20% of the 862 patients. The CFR varied from 7 to 12% and was lower than for patients with shock alone, but significantly higher than for patients with meningitis alone (Table 1) (6, 8, 9).

2.3. Meningitis Without Shock

This is the most common clinical presentation (6, 8–11) (Table 1). The symptoms develop more gradually than patients with shock with a median onset – admission time of 23–29 h in five European studies (6, 11, 19). After a comparatively low graded meningococcemia, the bacteria may transverse the blood–CSF barrier of meningeal blood vessels and enter the subarachnoid space and possibly also enter the lateral ventricles of the brain by passing through the choroid plexus (19). In general, meningococci proliferate in the CSF to levels that are 3–5 $\log_{10}$ -fold higher than the plasma levels. Approximately 70% of these patients have *N. meningitidis* DNA copy number $<10^3$ /mL in plasma. The median LPS level in plasma is <0.5 EU/mL (23). CSF may contain up to 10^9 /mL meningococcal DNA copies (23). The CSF concentration of LPS may reach 4,000 EU/mL (19). Concomitantly, bacteria trigger the release of a variety of inflammatory mediators including cytokines and chemokines, whose levels are several $\log_{10}$ -fold higher in CSF than the levels measured in plasma. The patients present with distinct clinical symptoms and signs of meningism, i.e., neck and

back rigidity, headache, vomiting, photophobia, and positive Kernig's and Brudzinski's signs. The CSF contains $\geq 100 \times 10^6/\text{L}$ leukocytes, an elevated level of proteins, and a lower than normal content of glucose. In the 862-patient cohort, 37–49% were classified as meningitis without shock. The CFR was $\leq 1\%$ (Table 1) and the major sequel was deafness (14).

2.4. Meningococemia Without Shock or Meningitis (Mild SMD)

The patients usually present with fever. They may have a petechial rash but lack signs of septic shock or meningitis. Blood cultures are positive in 70–80% of the cases (19). The meningococemia is occasionally transient and only present in the prehospital phase. *N. meningitidis* is detected in cultures from blood or biopsies(s) and PCR or ELISA (for research purpose). The median number of meningococcal DNA copies was $7.7 \times 10^3/\text{mL}$ and LPS level $< 0.5 \text{ EU}/\text{mL}$ in one study (23). If a spinal puncture is performed, the CSF is normal or shows signs of slight pleocytosis ($< 100 \times 10^6/\text{L}$ leukocytes). Given the fact that these patients do not develop long-lasting circulatory impairment or a massive inflammation in the subarachnoid space the CFR is low. When death does occur, it is related to the development of shock or brain edema after admission. This clinical presentation occurred in 18–33% of the 862 patients studied prospectively with a CFR of 0–5% (Table 1). It is a composite clinical group. Untreated, some of these patients might have developed shock or meningitis later, whereas others are transient meningococemia possibly seeding joints, pericardium, or eyes (4, 14). Very few develop subchronic or chronic meningococemia with intermittent fever and rash (4, 11, 14). In the Netherlands, the CFR among 752 prospectively studied patients from 2003 to 2005 was inversely related to the duration of the disease before admission (Table 4) (6). The longer the duration of the symptoms the lower the proliferation rate of meningococci in the circulation.

3. *N. meningitidis* Interacting with the Human Innate Immune System

The disease severity and inflammatory response are closely associated with the level of LPS in plasma and CSF, reflecting the growth of meningococci in different compartments (10, 19, 23, 28, 29). In all four clinical presentations described earlier, meningococci can be grown in both the blood and CSF and the levels of bacteria in the two compartments determine the clinical symptoms (19, 23) (Table 5).

Lipopolysaccharides (LPS, endotoxin) are the principal, but not the only molecules, in the outer membrane of the meningococcus leading to a generalized activation of the innate immune system (11, 19, 23, 28–32). The immunological response is reflected

Table 4
Inverse relationship between case fatality rate (CFR)
and duration of the disease before admission (6)

Duration	<12 h (%)	12–18 h (%)	18–36 h (%)	>36 h (%)
Case fatality rate	10.2	7.8	3.5	2.2

Table 5
The percentage of positive cultures of blood (157 patients)
and CSF (119 patients) with microbiologically confirmed
diagnosis of SMD before receiving antibiotics (19)

	Shock (%)	Shock + meningitis (%)	Meningitis without shock (%)	Mild meningococ- cemia (%)
Blood	93	87	50	77
CSF	59	83	84	47

by cytokine levels, release of other inflammatory mediators, activation of complement, and coagulation and inhibition of the fibrinolytic systems (11, 19). Non-LPS molecules do activate the innate immune system, albeit less strongly than LPS. Porins (PorB) and presumably other lipoproteins have been reported to activate Toll Like Receptor (TLR) 2, meningococcal DNA activates TLR9 through CpG repeats, and fragments of peptidoglycan may activate NOD1 and NOD2 (33–37). The role of the non-LPS molecules in human disease is still unknown.

3.1. Structure of *N. meningitidis* LPS

Meningococcal LPS is often referred to as lipooligosaccharide (LOS) owing to a short side chain typical for Gram-negative bacteria found in the upper respiratory tract. It consists of lipid A, a core structure comprising two 2-keto-3-deoxy-octulosonic acid (KDO) and two heptoses (L-glycero-D-manno-heptopyranoside) substituted with short polysaccharide side chain (29, 38, 39). Lipid A and KDO represent the toxic moiety, i.e., the immune stimulatory part activating TLR4 when in complex with myeloid differentiation factor (MD)2 (40). The full length side chain comprises lacto-N-neotetraose (Gal β 1 $\rightarrow$ 4GluNAc β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Glc) and a terminal sialic acid. Lacto-N-neotetraose and sialic acid play a role in avoidance of immune recognition (molecular mimicry), serum resistance, and cell adherence. Based on antibody specificity, 12 different LPS immunotypes have been described. Immunotypes 3, 7, and 9 are found in approximately 80% of isolates from Dutch patients and represent LPS with a maximum number of sugars

including a terminal sialic acid (41). Carrier isolates often harbor a shorter LPS (L8) making them more adhesive, but vulnerable to bactericidal antibodies and less virulent (29).

3.2. Variation in the *N. meningitidis* LPS Lipid A Structure Is Reflected by a Reduced Capacity to Induce Cytokines

Mutated strains of *N. meningitidis* comprising four or five fatty acids in the lipid A moiety, as opposed to hexavalent lipid A, are less biologically active than the wild-type parent strains (42). Wild-type strains with penta-acylated lipid A may infect patients (43). They apparently cause infections with less activation of the coagulation system than the hexa-acylated wild-type strains usually isolated from patients. The ability of *Neisseria* isolates to activate the LAL assay differs significantly and is presumably related to certain variations in the lipid A structure, including variable phosphorylation of the backbone structure (44).

3.3. Minimal Chain Length Requirement of *N. meningitidis* LPS for Optimal TLR4-MD2 Activation

Based on studies of *E. coli* lipid A it was assumed for a long time that lipid A derived from meningococci represented the toxic moiety of the molecule. However, by systematically testing the biological activity of *N. meningitidis* mutants expressing variable lengths of the polysaccharide side chain and core region of LPS, it has been documented that lipid A per se is less active than lipid A to which two KDO molecules are attached (40). Synthetic lipid A containing one KDO is markedly more potent than lipid A alone (45). MD2 in complex with lipid A is crucial for TLR4 activation (46).

3.4. Studying the Activation of Complement and Different Cell Types by Wild Type and LPS-Deficient *N. meningitidis*

Two international reference strains (H44/76 and FAM20) have been transformed into viable mutants completely lacking LPS (47, 48). By systematically comparing the effects of the LPS-containing wild-type strain with the LPS-deficient mutant (H44/76 *lpxA*⁻) in various cell lines, animal models, and in whole human blood models, the contribution of LPS in the inflammatory response has been clarified (49). A general conclusion is that LPS in *N. meningitidis* is the most potent group of molecules for inducing inflammation in the host. Ten to 100 times higher numbers of mutant bacteria are required to induce inflammatory responses of the same magnitude when assayed in human whole blood models, different cell lines, and mice (31, 32, 49).

3.4.1. Complement Activation vs. Load of *N. meningitidis*

Studies of complement activation in patients revealed a dose-dependent association between the levels of LPS and formation of the terminal complement complex (C5b-polyC9, TCC) in plasma, the clinical presentation and lethal outcome (50). Subsequent studies documented that complement activation induced by wild-type meningococci and the LPS-deficient mutant was independent of surface exposed LPS (51). LPS in patient plasmas served merely as a marker of other meningococcal molecules activating complement. In vitro experiments showed that 3×10^7 /mL *N. meningitidis* in serum was required to generate significantly increased levels of

TCC, indicating terminal pathway activation (31). Looking back at the patient data, the marked elevation of TCC in EDTA-plasma was associated with high-grade meningococemia and LPS levels ≥ 100 EU/mL, which is roughly equivalent to $\geq 10^7$ /mL copies of *N. meningitidis* DNA and an 85% CFR (23, 50).

3.4.2. Interaction of Toll-Like Receptors and Complement in Human Whole Blood, Simulating Sepsis

By simulating the log phase growth of *N. meningitidis* to fulminant sepsis it has been documented that TLR4 is activated with much lower levels of meningococci than are required to activate the complement system with significant increase of TCC. The difference was in the magnitude of 3–4 $\log_{10}$ (31). However, blocking experiments using whole blood triggered by increasing doses of meningococci documented a synergy between TLR4 and complement activation. The cytokine production was augmented with much lower levels of meningococci than were required to induce TCC as measured by ELISA, which indicated low-grade complement activation (31).

3.4.3. *N. meningitidis* Activating Human Monocytes

Human monocytes are precursors of tissue macrophages in liver, spleen, lungs, peritoneum, and other tissues. They are key cells in the mononuclear phagocyte system. In a comparative study using microarray to evaluate the influence of meningococcal LPS vs. non-LPS structures on gene regulation, 4,689 monocyte genes were either twofold up-regulated or down-regulated by 10^6 /mL of wild-type *N. meningitidis* strain H44/76 (52). Only 72 genes were differentially regulated by 10^6 /mL of LPS-deficient mutant bacteria. By increasing the numbers of LPS-deficient mutant bacteria to 10^8 /mL, the expression of 3,905 genes was altered, indicating a dose-response activation of the monocytes induced by a 100-fold higher number of non-LPS molecules. Further analysis revealed that 2,288 genes were particularly LPS sensitive: these genes were up- or down-regulated by 10^6 /mL wild-type meningococci but not by 10^8 /mL LPS-deficient mutant bacteria (52). The same quantitative pattern of cytokine production is present in human whole blood models where increasing numbers of wild-type *N. meningitidis* H44/76 are compared with the LPS-deficient mutant (31).

3.4.4. *N. meningitidis* Activates Endothelial Cells

Current hypotheses suggest that meningococci are most likely to enter the subarachnoid space between or through endothelial cells of blood vessels located within the meninges (53–55). Using different tissue culture models simulating the interaction between *N. meningitidis* and human brain microvascular endothelial cells, a significant reprogramming of gene expression occurred in the host cells (53, 56). Key mRNA transcripts for cytokines and chemokines including TNF- α , IL-6, and IL-8 were up-regulated in the cells. ICAM-1 mRNA was likewise up-regulated, facilitating the transition of activated neutrophils through the capillary wall. IL-1 β was unchanged. Expression of pili increased gene expression, whereas

the presence of capsule reduced expression (53). The cells also showed signs of antibacterial resistance by activating antiapoptotic mechanisms (56).

3.4.5. Intracellular
Signaling Related
to LPS and Non-LPS
Meningococcal Molecules

Transmembrane signals generated by wild-type meningococci are conveyed via myeloid differentiation primary response protein (MyD)88-dependent and -independent pathways (52, 57). Genes for cardinal cytokines including TNF- α , IL-1 β , IL-6, IL-8, and MCP-1 are activated via the MyD88 pathway primarily by LPS, but also with very high levels of the mutant, by non-LPS molecules (31). The large numbers of particular LPS-responsive genes are primarily activated by MyD88-independent mechanisms via increased levels of interferon- β , which activates interferon- β inducible genes (type I interferon- β signaling pathway) (52). NO, a powerful vasodilator contributing to the shock, is induced via the MyD88-independent pathway (57).

**4. A New Porcine
Model Simulating
Fulminant
Meningococcemia
with Profound
Shock**

Stepwise increasing doses of heat-killed wild-type serogroup B meningococci (H44/76) or LPS-deficient (H44/76 *lpxA*-) strains were infused into anesthetized healthy landrace pigs, simulating the logarithmic growth of meningococci in patients with shock (58, 59). Pulmonary hypertension induced by thromboxane A₂, endothelin, and other mediators were the first significant changes in the cardiovascular system. They were induced earlier and more intensively by the wild-type strain than the LPS-deficient mutant but finally reached the same level. In parallel, a general vascular leakage occurred. Subsequently, reduced vascular resistance and decreasing mean arterial pressure followed in the animals given wild-type meningococci but not in the pigs receiving the LPS-deficient mutant (59). Animals challenged by the wild-type strain but not by the mutant lacking LPS became profoundly leukopenic, indicating “margination” of leukocytes in the vasculature. The leukopenia indicated up-regulation of various adhesion molecules on the leukocytes and the endothelial cells by LPS. The wild-type strain activated the coagulation system more profoundly than the mutant. This model confirmed the potent immunostimulatory role of LPS as the major sepsis-inducing molecule of meningococci. However, the non-LPS molecules of the mutant were fully capable of inducing TNF- α , IL-6, and IL-10 but not IL-1 β , IL-8, and IL-12. The wild-type strain was approximately ten times more potent than the LPS-deficient mutant (59). Massive complement activation resulting in increased TCC in plasma did not occur since the levels of meningococci in the blood were below 10⁷ *N. meningitidis* DNA copies/mL (31, 59).

5. Conclusion

The inflammation induced by *N. meningitidis* has been studied in patients and in various experimental models. LPS is crucial, but not the only molecule capable of activating the innate immune system. Rapid intravascular proliferation of meningococci leading to massive endotoxemia and shock is the greatest threat to the patient. Proliferation in the subarachnoid space leading to distinct meningitis has a much better prognosis if the patient is treated with appropriate antibiotics. Why as many as 30% of patients with SMD develop massive meningococcemia leading to septic shock and multiple organ failure is presently not understood and not observed with any other human invasive bacterium.

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