Review

Neisseria meningitidis: pathogenetic mechanisms to overcome the human immune defences

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Key words

Neisseria meningitidis • Immune-system • Virulence • Pathogenetic factors • Vaccines

Summary

Neisseria meningitidis is hosted only by humans and colonizes the nasopharynx; it survives in the human body by reaching an equilibrium with its exclusive host. Indeed, while cases of invasive disease are rare, the number of asymptomatic Neisseria meningitides carriers is far higher. The aim of this paper is to summarize the current knowledge of survival strategies of Neisseria meningitides against the human immune defences.
Neisseria meningitidis possesses a variety of adaptive characteristics which enable it to avoid being killed by the immune system, such as the capsule, the lipopolysaccharide, groups of proteins that block the action of the antimicrobial proteins (AMP), proteins that inhibit the complement system, and components that prevent both the maturation and the perfect functioning of phagocytes. The main means of adhesion of Neisseria meningitides to the host cells are Pili, constituted by several proteins of whom the most important is Pilin E.
Opacity-associated proteins (Opa) and (Opc) are two proteins that make an important contribution to the process of adhesion to the cell. Porins A and B contribute to neisserial adhesion and penetration into the cells, and also inhibit the complement system.
Factor H binding protein (fhbp) binds factor H, allowing the bacteria to survive in the blood.
Neisserial adhesin A (NadA) is a minor adhesin that is expressed by 50% of the pathogenic strains. NadA is known to be involved in cell adhesion and invasion and in the induction of proinflammatory cytokines.
Neisserial heparin binding antigen (NHBA) binds heparin, thus increasing the resistance of the bacterium in the serum.

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Introduction

Neisseria meningitidis is a gram negative bacterium which is hosted solely by the man. Usually, the microrganism, that finds its habitat in the nasopharynx, can live with the human body without causing damage. Indeed, *N. meningitidis* to adapt itself to survive in human body should find equilibrium with its exclusive host. In other terms, meningococcus should pursue the aim to reach almost a symbiotic coexistence with the humans. Indeed, the disease is almost an exception, and the rule is that the bacterium survives in rhinopharynx for a limited period of time. Consequently the number of carriers of meningococcus is very higher than the number of meningitis cases.
However, sometimes, because of its own characteristics of virulence or because of weak conditions of the human body, meningococcus can cause devastating consequences, such as meningitis, sepsis, and the Waterhause-Friederiksen's syndrome [1].
As other bacteria, in nature, meningococcus is continually subjected to unfavourable environmental conditions of life. The adaptation, under such difficult conditions, requires to meningococcus to implement many survival strategies [2].
The most important human defence against the bacterial colonization is the immune-system. Indeed, the innate immune response ensures immediate protection irrespective by the antigen, and it is realized through epithelial and phagocytic cells, together with the complement and substances with antimicrobial action. Later, when adaptive immunity also operates, the bacteria are cleared [3]. Infants come in contact early with different members of Neisseriaceae family bacteria, and the first contact is the more dangerous [4]. Subsequent contacts help the infant to mount an immunological response, which has several fluctuations during the infancy and childhood. The antibodies decrease during the adolescence [5], and, in the same time, the social life of the young facilitates the circulation of *N. meningitidis*. In the young the pathogen arranges the fulcrum for its survival. Indeed, in the young it is possible to find the highest percentages of carriers [6].
A genome very compact but extremely variable allows meningococcus to circumvent the immune defences. N. menigitidis has the ability to acquire genes of other bacteria from the environment and so generate new variants [7].
Another important defence mechanism of the meningococcus is its ability to camouflage some surface structures, making them similar to substances peculiar of the human organism. This feature is particularly evident for the meningococcus of serogroup B [3].

N. meningitidis interactions with the nasal mucosa cells

The most important route of meningococcal transmission is the contact from person-to-person through Flügge's droplets from asymptomatic carriers or from persons with invasive disease.
Once arrived in the nasal mucosa, meningococcus probably uses various defence mechanisms, such as to remain aggregated to reduce the exposure of surface antigens and to produce a lot of outer membrane vesicles (OMV) to trick the human body's defences, as IgA secretory antibodies.
At the level of epithelial barrier meningococci should escape several immunological defences, in particular: antimicrobial compounds, as for instance peptidoglycan recognition protein, proteins involved in iron metabolism, etc, and oxygen and nitrogen species (ROS and RNS) produced by activated phagocytic cells. Furthermore, although less abundant than in systemic circulation, complement factors are present also on the surface of the mucosal cells.
Although the mechanism of action of antimicrobial compounds is not yet completely known, however it is probable that these proteins act modifying bacterial membrane stability and permeability. Antimicrobial proteins and peptides also bind to lipopolysaccharide (LOS/LPS) of Gram negative bacteria, thus neutralizing their endotoxic activity [3] (Fig. 1).
Subsequently, Neisseria must avoid mucus clearance, and so must adhere to the epithelial cells. For this goal meningococcus has several mechanisms of action. The pili are the most important Neisserial adhesins, which are constituted by several proteins. The most important is Pilin E (PilE) [8, 9], however two distinct minor pilus-associated proteins, PilC [10], and Pil Q [11] have been recently described. The pili proteins are very variable. The cellular receptors for the interaction with Neisserial pili are not completely known, however, probably they interact with membrane co-factor protein, also known as CD46 receptor, and with alpha 1 and alpha 2 integrins.
N. meningitidis, usually, expresses two kinds of outer-membrane proteins, the opacity-associated proteins Opa and Opc, which confer opacity to agar-grown colonies. Opa proteins can attach to carcinoembryonic antigen-related cell-adhesion molecule (CEACAMs), which belong to a family of the immunoglobulin superfamily [12, 13] and HSPGs (heparan sulfate proteoglycans) [14] and Opc proteins can interact with HSPGs and, through the vitronectin and fibronectin, to their integrin receptors.
Further, minor adhesins can help the meningococcus to adhere to epithelial cells. These adhesin are: Neisserial Adhesin A (NadA, that is expressed by 50% of the pathogenic strains, but only by 5% of the strains isolated from carriers); an OCA (oligomeric coiled-coil adhesin); NhhA (Neisseria hia homologue A) and App (adhesion and penetration protein), expressed by virulent *N. meningitidis* strains, whereas MspA (meningococcal serine protease A), that is an homologous of App, is only sometimes expressed by virulent strains [8].

Penetration and colonization of the respiratory mucosa

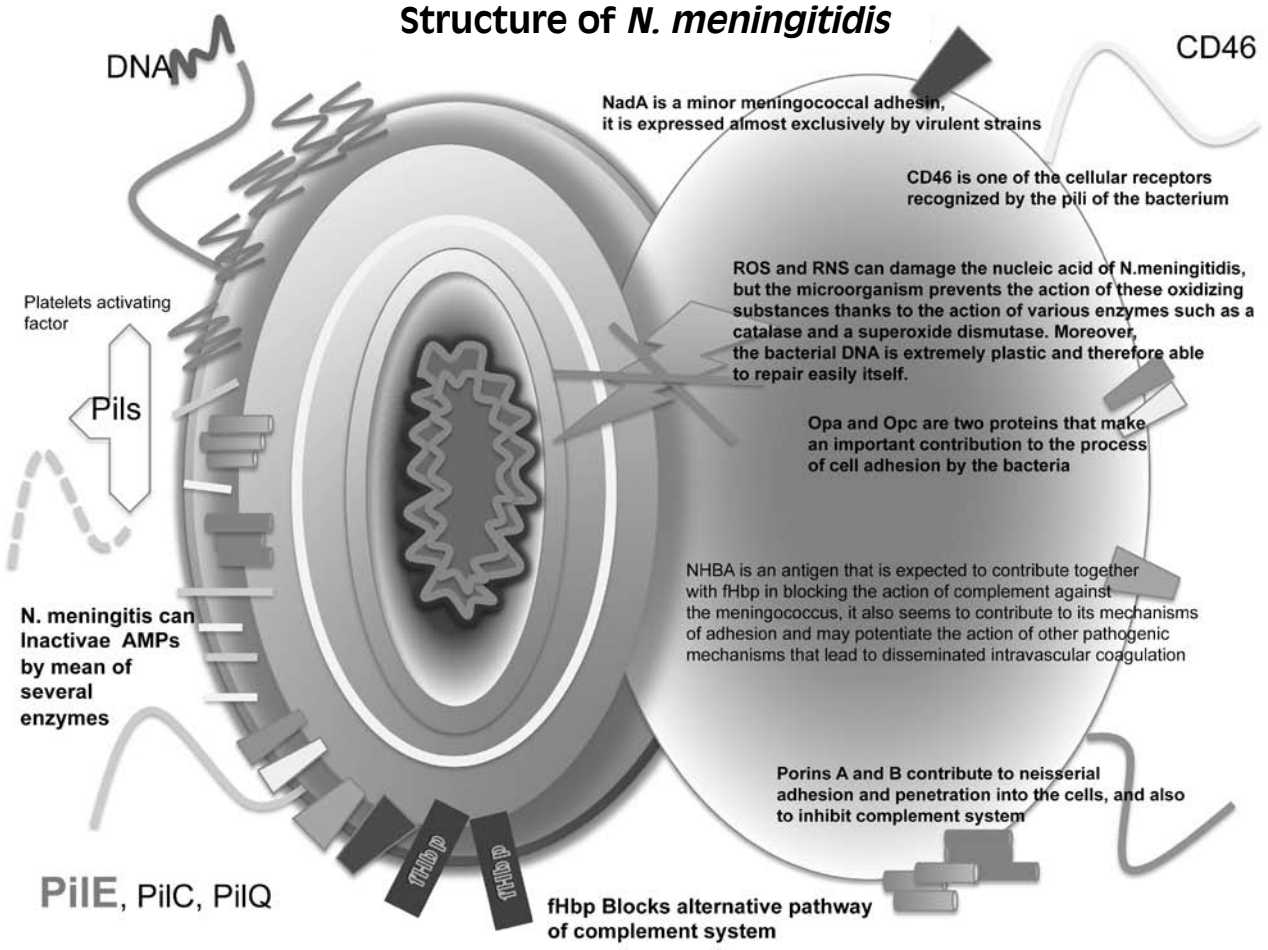
Recently, it has been demostrate that Opc is able to bind to the α-actinin cytoskeletal protein of both epithelial and endothelial cells [15]. To cross the epithelial barrier, meningococci interact with the extracellular matrix proteins, both the fibronectin (Fn) and the vitronectin (Vn). Furthermore, it is possible that minor adhesins are able to help bacterial invasion of the mucosal barriers.
When *N. meningitidis* reaches sub-epithelial tissues is widely exposed to immunologic cells. In particular to dendritic, macrophage, and neutrophil cells.
Dendritic cells (DCs) have important functions in antigen presentation and in immune-homoeostasis [16]. These cells are also very important to initiate adaptive immune responses. Both at mucosal surfaces and, overall, in sub-epithelial tissues, these cells govern responses both to pathogenic and commensal bacteria. They have a crucial importance in the mechanisms for distinguish microrganisms that must eliminating or tolerating [16]. Activated DCs secrete many cytokines, as: IFNγ and IL-12 (which activate the Killer cells; IL-12, also, activate naïve CD4- cells), IL-6 and 23 (which stimulate Th1 cells to secrete IFNγ, TFN, and lymphotoxins; IL-6, also, activate Th2 cells to secrete IL-4, IL-5, IL-13 and IFN-alpha). As other cells of the immune system DCs can be activated via Toll-Like-Receptors (TLRs). Until now, at least TLR 2, 3, 4, 5, 6, 7, 8, 9 and 10 have been identified on the surface of DCs. Further, also macrophages have many TLRs. In particular TLR 4 appears to be activated by Neisserial antigens, especially by lipo(oligo)saccharide (LOS). After the activation via TLR4, macrophages release: cytokines, chemokines, nitric oxide, and ROS.
Experiments in vitro have shown that dendritic cells are able to kill wild-type *N. meningitidis* strains [17]. However, it has been demonstrated that encapsulated, wild-type *N. meningitidis* have a lower capacity to adhere to dendritic cells than unencapsulated strains [17, 18]. Further, lipopolysaccharide sialylation inhibits phagocytosis [18, 19]. Thus, even if capsule expression is low, the bacterium could be still protected from dendritic-cell phagocytosis [3]. More recently, it has been demonstrated that live meningococci is able to interfere with maturation of DCs [20]. Thus *N. meningitidis* can prevent the development of an effective cellular and humoral immune response. Furthermore, in the lamina propria of the mucosa, meningococcus is able to escape from possible damages by ROS and RNS by a group of enzymes, such as, for instance: a katalase, an oxide reductase, a superoxide dismutase and glutathione peroxidase. At this level, Neisseria must fight also against substances secreted by neutrophil cells. These cells, in addition to producing antimicrobial peptides, as beta-defensins, can secrete other compounds as: elastase, lactoferrin, and lysozyme [21] *N. meningitidis* exploits many stratagems to evade the actions of these antimicrobial molecules, among which is worth citing also LL-37 peptide, which is secreted by macrophage, and which is the unique cathelicidin known until now. Between the

different components of the outer membrane, lipopolysaccharide plays an important role in protecting the bacterial cell. Indeed, in particular, through a slight change in its chemical composition and therefore in its steric conformation, it can escape to neutralization action of antimicrobial compounds and so it can help to confer Neisseria resistance against antimicrobial peptides and proteins. However, the major system of bacterial defence is the capsule. Probably, the capsule due to its steric conformation prevents the antimicrobial peptides to reach the outer membrane of the bacterium. Remarkably, *N. meningitidis* also has an efflux pump, that appears very important for antimicrobial-protein resistance, since, as already said, antimicrobial proteins appears to act both modifying bacterial membrane stability and permeability and further inside the bacterial cells.
In addition, *N. meningitidis* secretes 2 proteins involved in iron metabolism, which is particularly important for bacterial growth. These 2 proteins, lactoferrin binding proteins A an B, are able to subtract the chelated iron from lactoferrin, making it available for the vital needs of the bacterium.

How *N. meningitidis* spreads and can survive in the bloodstream?

The same receptors that the meningococcus uses to adhere to epithelial cells of the respiratory mucosa are, probably, used by the microorganism to cross the endothelium from the capillaries, and invade the bloodstream.
In the bloodstream the microorganism is widely exposed to numerous immune mechanisms of defence, especially to the complement system. In particular, the production of proinflammatory cytokines produced by phagocytes, cause an inflammation which increases the chances of the bacterium to cross the endothelial barrier.
The complement system can be activated through 3 pathways. All the mechanisms of activation of the complement system converge in the nodal point of covalent attachment of C3b factor. C3b activation allows to reach three goals, such as: the processing antigen, for adaptative cellular and humoral immunity, C5b-9 activation, for disruption of bacterium membrane, and phagocytosis, for pathogen killing [22].
Each of the 3 pathways has its own specific focus. The classical pathway has its primary target in the antigen-antibody complexes, but also it recognizes certain pathogen-associated molecular patterns (PAMPs) as polysaccharides on the surface of microorganisms. The primary target of alternative pathway is any non-self molecular pattern of the human body. The mechanisms of recognition is based on a family of proteins named factor H. However, recently, it has been demonstrated that the protein named properdin is able to activate the alternative pathway, also when it is necessary to destroy apoptotic and necrotic human cells [23].
The last mechanism of complement activation is the Lectin pathway, which acts through several proteins, as, for instance, MBL (mannose-binding lectin) and ficolins [24].
Thanks to several mechanisms, *N. meningitidis* can escape from the complement system. One of these is its molecular mimicry of human structures (for instance, the meningococcus B polysaccharide capsule is constituted by poly-sialic acid, which is present in many mammalian organs during development), in this way correspondent antibodies do not can be produced and the classical pathway do not can be activated [25]. The capsule is the most important bacterial structure in the prevention of bacterium lysis, mediated by the complement system and by phagocytosis.
Further, *N. meningitidis* secerns a lot of blebs, containing outer membrane components, lipopolysaccharide included. In this way the microorganism can steer away from its self the defensive mechanisms, including the complement system [26].
Lipo-polysaccharide is a part of all Gram-negative bacteria, and it is fundamental in the machinery of the bacteria to resist to complement action [27].
In addition, *N. meningitidis* expresses a protein, fHbp (factor H binding protein) that captures the factor H, and consequently can block the alternative pathway activation. Factor H binding protein is a lipoprotein expressed on the surface of the microorganism [28]. Further, another surface protein, called neisserial surface protein A (NspA), able to bind human factor H (fH) has been detected [29].
Another protein which can help meningococci to escape from complement system is a lipoprotein named NHBA (Neisserial Heparin Binding Antigen) [30]. Although the function of this protein it is not yet completely known, however, this antigen expressed on the surface of the most part of *N. meningitidis* appears to capture heparansulfate molecules contributing to defend the bacterial cells against complement system. Furthermore, it is possible that NHBA contribute to the adesion mecchanisms to human cells, indeed OPC mediates the adhesion and invasion by vitronectin, and integrins, and mediation with vitronectin requires heparin.
In the bloodstream *N. meningitidis* stimulates cytokine release from phagocytic, lymphocytic and endothelial cells. One of the consequences of this release is the damage that leads to clinical symptoms, and, particularly to petechial rash. LPS, too, contributes to endothelial damage [31] (Fig. 1). LPS can also contribute to the activation of the coagulation system, up regulating the tissue factor. Abnormal activation of the coagulation system can provoke a disseminated intravascular coagulation (DIC), and the Waterhause-Friederiksen's syndrome [32]. It is possible that the DIC could be facilitate by NHBA too, which captures glycosaminoglycans (e.g. heparan sulfate) [30].

Fig. 1. The main components of the structure of *N. meningitidis* are:
Capsule: It is composed of polysaccharides containing sialic acid, which favor the mimicry which weakens the immune response;
The lipo-oliogosaccaride (LPS): The LPS is extremely variable. LPS can bind sialic acid, which helps the camouflage of the meningococcus. Lipoligosaccaride plays a crucial role in determining meningococcal sepsis. The LPS induces the formation of various cytokines that cause vascular endothelial damage, leading to necrosis and severe damage in many organs and systems. LPS causes the release of IL6 and TNF, ROS and NO, acting also through the TLR-4;
Lactoferrin binding proteins A and B: Neisseria expresses two superficial proteins named lactoferrin binding proteins A and B, which can bound iron which is an essential growth factor during bacterial colonization;
Porins: PorA and PorB are part of neisserial outer membrane and contribute to the mechanism of adhesion of bacteria to the cells, porin A also bind a regulatory protein of the complement;
Pili: The pili are the most important mechanism of adhesion of Neisseriae to the cells. The most important of the 3 known pilins is pilin E;
Opa and Opc: Neisseriae express 2 types of outer-membrane proteins, which give opacity to the colonies of meningococcus growth in agar plate. These proteins are involved in adhesion mechanisms, recognizing the CEACAM receptor. Opa also recognize another surface receptor HSPGs. OPC also mediates the adhesion and invasion by vitronectin, and integrins. Mediation with vitronecnin requires heparin;
Minor adhesins: Other adhesins are: Nad A, OCA, NhhA and the APP;
fHbp: fHbp bounds complement factor H, and in this manner promotes the resistance of the organism in the blood stream.



How *N. meningitidis* reaches Leptomeninges?

N. meningitidis can located it self in the leptomeninges adhering both to endothelial cells and to cuboid cells of choriod plexus. Low blood flow facilitates the adhesion, that is mediated by pili system [8]. However, also, Opa proteins, and especially, Opc, can contribute to adhesion and penetration of meningococcus into the cells of the leptomeninges [33].

It is important to note that, probably, the most important mechanism of crossing the Blood-Brain-Barrier (BBB) is the passage of meningococci through the junctions of endothelial cells [34], and the process of internalization of the bacterium in the endothelial cells (transcytose), although demonstrated, plays a minor role. Indeed, particularly, pro-inflammatory cythokines induced by LPS, through a cytopathic effect on endothelial cells, can help meningococci to cross the BBB, probably and overall, through the disruption of intercellular junctions. Higher

levels of IL-6 and -8, and a low level of chemokines (for instance CCL5), were found, in experimental studies to favour the adhesion of *N. meningitidis* on meningioma cells, comparing it with *N. lactamica* [35, 36].
Probably, also, minor adhesins are involved in the mechanism of adhesion and penetration of the bacterium in endothelial and in pia mater and arachnoid cells.

Conclusions

In conclusion, *Neisseria meningitidis* has developed a formidable machinery to survive in its unique biological niche that man. Five fundamental pillars support this adaptation, ie the capsule, the lipopolysaccharide, the groups of proteins that allow to block the action of the antimicrobial proteins, proteins that inhibit the complement system and components that prevent both the maturation and the perfect functioning of phagocytes. If the capsule and LPS appear as the main features of hypervirulent strains, other mechanisms allow the bacterium to survive, albeit for short periods, as a commensal in the nasopharynx. If the first contacts with the meningococci are the most dangerous, because they can more easily lead to illness, the particular habits of young people associated with a decline in immune protection typical of their age, make that young people are the main reservoir of *Neisseria meningitidis*.

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