Mechanism of meningeal invasion by Neisseria meningitidis

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Keywords: Neisseria meningitidis, cerebrospinal meningitis, blood-brain barrier, central nervous system infections, beta 2 adrenergic receptor, brain endothelial cells

The blood-cerebrospinal fluid barrier physiologically protects the meningeal spaces from blood-borne bacterial pathogens, due to the existence of specialized junctional interendothelial complexes. Few bacterial pathogens are able to reach the subarachnoidal space and among those, Neisseria meningitidis is the one that achieves this task the most constantly when present in the bloodstream. Meningeal invasion is a consequence of a tight interaction of meningococci with brain endothelial cells. This interaction, mediated by the type IV pili, is responsible for the formation of microcolonies on the apical surface of the cells. This interaction is followed by the activation of signaling pathways in the host cells leading to the formation of endothelial docking structures resembling those elicited by the interaction of leukocytes with endothelial cells during extravasation. The consequence of these bacterial-induced signaling events is the recruitment of intercellular junction components in the docking structure and the subsequent opening of the intercellular junctions.

Introduction

Bacterial meningitis is the leading cause of central nervous system (CNS) infection. Although bacterial meningitis can be due to the dissemination of contiguous infections such as sinusitis or mastoiditis to the meningeal membranes, most cases are caused by blood-borne pathogens. The blood-brain barrier (BBB) protects the CNS from most bacteria that may have reached the bloodstream, thus restricting the etiology of bacterial meningitis to a few and predominantly extracellular pathogens: Escherichia coli K1 and Streptococcus agalactiae (Group B Streptococcus) in the newborn, Neisseria meningitidis, Haemophilus influenzae type b and Streptococcus pneumoniae in children and adults.1–3 Paradoxically, these bacteria are commensal of the nasopharynx (N. meningitidis, S. pneumoniae and H. influenzae) or of the digestive tract (E. coli and S. agalactiae).4

The pathophysiology of bacterial meningitis is a multistep process that reflects the ability of bacterial pathogens to cross the oropharyngeal or digestive mucosal barrier, survive and replicate in the bloodstream, and to eventually cross the BBB.5–9 It has been demonstrated that these extracellular pathogens express various virulence factors allowing them to survive in the extracellular compartments and to interact directly with the components of the blood-brain barrier. The main virulence factor expressed by all extracellular pathogens is a capsule that prevents bacterial phagocytosis or complement-mediated lysis.4,7–12 Once inside the cerebrospinal fluid (CSF), bacterial multiplication is thought to be uncontrolled, due to the local deficiency in complement and immunoglobulins, despite the influx of polymorphonuclear leukocytes induced by the local inflammatory response. However data obtained in primates showed that bacterial presence in the CSF can be transient if bacteremia is not sustained, reflecting the fact that bacterial entry into the CSF may not always lead to meningitis.13

The small number of bacterial species capable of invading the meninges suggests that specific virulence factors are required for bacteria to enter the subarachnoidal space. Among the above-mentioned extracellular bacteria, Neisseria meningitidis, the agent of cerebrospinal meningitis, is the pathogen that once in the bloodstream is able to invade the meninges the most constantly. It has been estimated that 63% of bacteremia due to N. meningitidis are associated with meningitis.14 In this review, Neisseria meningitidis will be used as a model to address the mechanisms by which a bacterial pathogen can cross the BBB and invade the meninges.

The Cerebrospinal Meningitis

Neisseria meningitidis is a frequent asymptomatic colonizer of the human nasopharynx, and only a very small proportion of infections proceed to a sustained bacteremia. Once in the bloodstream N. meningitidis can either be responsible for a deadly septic shock leading to purpura fulminans and/or cross the BBB to invade the meninges. The reasons why disease occurs in some individuals and not in others remains unclear, but human genetic polymorphism is likely to be important in determining the outcome of infection.15,16 In addition, all meningococci do not have the same pathogenic potential. Indeed, analysis of results from multilocus sequence typing (MLST) has demonstrated the existence of distinct phylogenetic groups (clonal complexes), some of which are more likely to be isolated from patients than others.17
These are the so-called “hyper-virulent” or “hyperinvasive” lineages. Recently, the presence of a prophage has been shown to be responsible for a large proportion of invasiveness of strains belonging to hyperinvasive lineages.18,19 This element inserted into the bacterial chromosome can be induced to produce a filamentous phage.

* N. meningitidis * interacts only with human cells and there is no animal model of meningococcal sepsis. In some circumstances mice and infant rats have been used to assess the ability of the bacteria to survive in the extracellular fluids,20 but these models are unable to assess the consequences of the interaction with endothelial cells. Most of the hypotheses regarding the pathogenesis of meningococcal infections have been obtained studying postmortem samples and/or biopsies of skin lesions.11,21 * N. meningitidis * interacts with endothelial cells and form colonies on the apical surface of endothelial cell capillaries. Bacteria are also found inside cells and in intercellular spaces. In peripheral purpuric lesions, retraction of endothelial cells with capillary disruption can be observed, as well as hemorrhages, adhesion of leukocytes and formation of small thrombi. In the brain, bacteria are seen interacting with capillaries of the subarachnoidal space, the brain parenchyma and the choroidal plexuses, and inside brain vessels. When a low or moderate number of meningococci is present in the bloodstream, the bacteria interacting with peripheral capillaries cause only few localized purpuric lesions, whereas the interaction with brain endothelial cells is sufficient to lead to meningeal invasion. Adhesion of the bacteria to the meninges and meningeal cells,22 is then probably critical for * N. meningitidis * to disseminate through the meningeal spaces. In contrast, in case of high bacteremia, many peripheral endothelial cells are colonized by meningococci, leading to a significant increase of vascular permeability possibly associated with extensive thrombosis and purpura.

**Where is the Blood-Brain Barrier Breached?**

The blood-brain barrier is a highly specialized structural and functional component of the central nervous system that separates the circulating blood from the brain and spinal cord parenchyma. Among the different cellular types that make up the BBB, endothelial cells form the front defense line of the CNS parenchyma against invading pathogens. Schematically, the capillaries of the CNS parenchyma have two specific features that are not shared by those of other peripheral organs: (1) the presence of specialized junctional complexes and (2) a sparse pinocytotic vesicular transport activity that is counterbalanced by highly specialized transport systems that limit the entry of neuroactive blood-borne molecules.23 Junctional complexes are composed of adherens junctions and of multistranded belts of tight junctions that result in apparent membrane fusion of adjacent endothelial cells, forming a continuous blood vessel. Interendothelial tight junctions exclude the paracellular passage of hydrophilic macromolecules between the blood and the brain and account for the high endothelial electric resistance of brain capillaries.24,26 Recently the endothelial Wnt/β-catenin signaling pathway has been shown to regulate the induction and maintenance of the BBB during embryonic and postnatal development.27,28 In vitro, the specialization of brain capillary tight junctions has been shown to be under the control of paracrine factors produced by astrocytic end-feet unsheathing brain capillaries. In addition to astrocytes, other perivascular cells including pericytes, brain macrophages and microglial cells form a perivascular immunological barrier of the CNS. Some recent data have underlined the role of pericytes in the regulation of the blood-brain barrier.29,30 On the arterial side of the brain capillaries, the perivascular space localized between endothelial cells and astrocytes is virtual and both basal lamina produced by astrocytes and by endothelial cells are in very close contact and can be fused. For this reason, the blood-brain barrier at the capillary level is also called the “gliovascular” or “neurovascular” unit. The gliovascular unit is a dynamic structure whose main function is to actively regulate brain homeostasis and protect the brain from circulating blood-borne insults.

Brain post-capillary venules, venules and veins are also part of the blood-CNS interface. The structure and function of the brain venous network strikingly differs from that of brain capillaries. Electron microscopy studies showed that when venules turn into veins and exit the brain parenchyma the interendothelial tight junctions are leaky.31-33 A possible explanation for this is that astrocytic end-feet require intimate interactions with endothelial cells to induce a “full” BBB phenotype.34,35 Indeed, when capillaries turn into venules, endothelial cells of the brain draining system are progressively separated from astrocytic end-feet by the Virchow-Robin perivascular spaces. The space between endothelial cells and astrocytes, designated as the Virchow space, is even more important when veins exit from the brain parenchyma into the subpial space. The structure of brain post-capillary venules and veins and their ability to express leukocyte adhesion molecules36,37 likely reflect their role in mediating the entry of leukocytes and plasmatic molecules in the perivascular space during inflammatory processes.35,38 These findings highlight that, probably due to different physiological functions, both the anatomy and the structure of these brains vessels differ, and that the venous system of the CNS can be considered as a relatively vulnerable localization of the blood-brain barrier.

The precise site and mechanism of entry of * N. meningitidis * into the CSF is still enigmatic. As shown from the postmortem examination of patients who died of meningococcal infection,21 extracellular bacterial pathogens can interact directly with the components of the blood-brain barrier and do not need a Trojan horse, such as leukocytes, to cross the BBB. Initial work suggested that bacteria may preferentially use the choroid plexus (CPs) route to cross the blood-CSF barrier. However, in this case, meningitis should theoretically be associated with ventriculitis, which is not corroborated by clinical data. Because of their proximity to the subarachnoidal space and their “leaky” interendothelial structure, the brain postcapillary venules and veins of the subpial and subarachnoid spaces may be the site of passage of bacteria into the CSF. Indeed, once bacteria have crossed the endothelial monolayer of these vessels, they could be easily drained into the CSF using the Virchow-Robin perivascular spaces. Interaction with the brain endothelial cells is therefore a crucial step in CNS invasion by * N. meningitidis. *
How is N. meningitidis Breaching the Blood-Brain Barrier?

Following the interaction of the bacteria with the endothelial cells, at least four strategies are possible for a microorganism to cross a monolayer of brain endothelial cells: (1) transcellular transport by passive or adhesion-induced transcytosis, (2) paracellular passage through opened tight junctions, (3) disruption of the endothelial barrier due to a direct cytotoxic effect and (4) leukocyte-facilitated transport by infected phagocytes.

These routes are not exclusive, as shown for viruses that may both directly interact with the blood-brain barrier or be transported by infected leukocytes to penetrate into the CNS.49 However and as already mentioned, extracellular pathogens probably do not use leukocytes as vehicles to cross the blood-CSF barrier. A breakdown of the blood-CNS barrier due to apoptosis or bacterial cytotoxicity is unlikely, since tissue lesions such as hemorrhages in the subarachnoid space are uncommon during bacterial meningitis. Therefore, the entry of blood-borne pathogens most probably respects the architecture of the blood-CNS barrier.12 Accordingly, adhesion of bacteria to endothelial cells can induce an intracellular signaling pathway leading to disruption of intercellular tight junctions or, alternatively, bacteria may induce their own transcytosis through the cell monolayer. In vitro, transcytosis of bacteria through human brain endothelial cells has been demonstrated for S. pneumoniae,40 E. coli,41-43 S. agalactiae44 and through human umbilical vein endothelial cells for H. influenzae.55,56 Although N. meningitidis can readily be internalized in vitro within vacuoles in human brain microvascular endothelial cells,47 recent data clearly demonstrate that N. meningitidis can open gaps in a monolayer of brain endothelial as a consequence of the delocalization of junctional components whereas in the same cell line transcytosis was not observed. These in vitro data suggest that N. meningitidis cross the BBB using the paracellular route. The molecular events elicited by meningococcal interaction leading to the opening of the paracellular route will be discussed below.

Bacterial Attributes Required for Meningeal Invasion

The growth/survival in extracellular fluids. The level of bacteremia is directly correlated with meningeal invasion by N. meningitidis. The bacteremia is believed to favor meningeal invasion by directly increasing the likelihood of the interaction of the bacteria with the components of the blood-CSF barrier. Therefore, the bacterial attributes involved in the growth and/or the survival in the extracellular fluids are playing an essential role in meningeal invasion by N. meningitidis.

Some of these virulence factors are commonly observed in most extracellular pathogens. They participate in the prevention of bacterial killing by the host effectors of the innate immune systems such as polymorphonuclear neutrophils and complement. These bacterial attributes are the polysaccharidic capsule, the lipoooligosaccharide (LOS), and iron chelation systems. A new virulence factor, the factor-H binding protein (fHBP), was recently identified.48 It is a 28 kDa surface-exposed lipoprotein that binds factor H, a key inhibitor of the alternative complement pathway. This protein is expressed by all N. meningitidis strains studied to date, although the level of expression varies between strains (high, intermediate or low expressers). Antibodies directed against fHBP are bactericidal and this protein is currently one of the best vaccine candidates.49 More recently, it has been shown that a mechanism by which N. meningitidis escape the killing by polymorphonuclear neutrophils may induce their own transcytosis through the cell monolayer. In vitro, transcytosis of bacteria through human brain endothelial cells has been demonstrated for S. pneumoniae,40 E. coli,41-43 S. agalactiae44 and through human umbilical vein endothelial cells for H. influenzae.55,56 Although N. meningitidis can readily be internalized in vitro within vacuoles in human brain microvascular endothelial cells,47 recent data clearly demonstrate that N. meningitidis can open gaps in a monolayer of brain endothelial as a consequence of the delocalization of junctional components whereas in the same cell line transcytosis was not observed. These in vitro data suggest that N. meningitidis cross the BBB using the paracellular route. The molecular events elicited by meningococcal interaction leading to the opening of the paracellular route will be discussed below.

The interaction with brain endothelial cells. As mentioned above, interaction with endothelial cells is essential in meningococcal pathogenesis. In vivo, blood flow generates mechanical forces that vary depending on the vessels and that could prevent bacterial interaction with the endothelial cells. The ability of N. meningitidis to bind to endothelial cells in the presence of shear stress mimicking the bloodstream was recently investigated.51 These data revealed that, after initial attachment, bacteria have the ability to resist high blood velocities, to multiply and to form microcolonies onto the apical surface of the endothelial cells. This resistance to shear stress and the ability to grow at the luminal surface of endothelial cells in the presence of blood flow highlight the efficacy of the interaction between N. meningitidis and the host cells, for which N. meningitidis has evolved efficient attributes.

Various bacterial surface components have been described allowing the interaction of N. meningitidis with human cells. These are type IV pili (Tfp) and other attributes such as Opa or Opc proteins (for review, see Carbonnelle et al.52,53).

However, in capsulated bacteria, type IV pili are the main bacterial attributes capable of promoting adhesion since non-piliated capsulated bacteria are unable to adhere to any cell type. Early work performed with piliated capsulated meningococci has shown that meningococcal interaction with human cells can be divided in two steps. The first one allows the adhesion of single diplococci in a rather inefficient manner. The second step corresponds to the bacterial division onto the apical surface of the cells. Therefore the high number of bacteria that interact with cells is a consequence of bacterial division of the few meningococci that have initially succeeded to adhere. Type IV pili are required for both steps. They promote the initial interaction of diplococci with the endothelial cells, and then generate bacteria-bacteria interactions that lead to the spreading of the bacteria on the apical surface of the cells.

Pilus biogenesis. Type IV pili are polymeric filaments found on many Gram-negative bacteria.54 These structures correspond to the multimeric assembly of a pilin subunit. Functional Tfp are dynamic structures. Pilin subunits are constantly assembled into filaments from a platform in the inner-membrane. The fiber is then extruded through the outer membrane via the secretin PilQ.54 A remarkable property of Tfp is their ability to retractor into the bacterium from which they originate, via the action of the force-generating ATPase PilT.55,56 Retraction is a consequence of
the disassembly of pilin subunits that are then stored in the cytoplasmic membrane. Tfp retraction is essential for bacterial motility (twitching motility), competence for DNA transformation and pilus-associated signaling to host cells.

The major neisserial pilus subunit, the pilin, is encoded by the pilE gene that is subject to antigenic variation following recombination with silent loci (pilS). Pilin is synthesized as a preprotein. A short leader sequence (5–6 residues) is cleaved by the prepilin peptidase PilD. PilD also methylates the N-terminal phenylalanine of the mature protein product. Pilins are packed through internal hydrophobic interactions between conserved N-terminal 2 helices, leaving hypervariable C-terminal globular regions exposed. PilE undergoes several post translational modifications of serine residues including glycosylation at position 63, whereas at position 68, the residue has been reported to be modified by phosphate, phosphoethanolamine or phosphorylcholine addition. Concerning the glycosylation, the structure of the sugar is different depending on the strain. In addition to PilE, pili also contain the low abundance proteins PilX, PilV and ComP. These are called minor pilins as they structurally resemble PilE and are likely to be assembled within the filaments in a similar way. Importantly, each minor pilin modules Tfp-linked properties. PilX is crucial for the formation of bacterial aggregates and indirectly controls adhesion to human cells by promoting bacteria-bacteria interactions. ComP is essential for competence for DNA transformation, while PilV affects several pilus-linked properties such as signaling to endothelial cells (see below).

The PilC proteins play a crucial but still enigmatic role. Two alleles were originally discovered. Expression of both variants is subject to phase variation as a result of frameshift in homopolymeric "G" tracts located in the open reading frames. PilC-null strains show impaired pilus expression and lack the ability for transformation competence. In N. meningitidis, only PilC1 is required for adhesion. PilC2, which is expressed independently of PilC1, fails to promote adhesion despite identical functions in pilus expression and transformation competence.

Abolition of pilT in a PilC-null background restores piliation, confirming the hypothesis that PilC acts as an antagonist of PilT by preventing PilT-mediated retraction.

Mechanism of pilus-mediated interaction with host cells. The molecular mechanism responsible for the first step of the adhesive process, i.e., the initial attachment of individual diplococci to the cells, is still not fully understood. One report suggested that the PilC1 protein could carry a cell binding domain. This hypothesis was based on inhibition of adhesion by purified PilC molecules. However, non-adhesive non-piliated isolates of a serogroup B strain with high PilC expression and pilated adhesive isolates with barely detectable PilC expression have been described. In addition, another PilC+PilE- strain, in which PilC location has been demonstrated in the outer membrane, is unable to interact with eukaryotic cells (Nassif X, unpublished observations), thus raising doubts on the role of PilC as an adhesin. It is therefore likely that the cell binding domain on the type IV pilin remain to be identified.

As mentioned above, the minor pilin PilIX is essential to promote inter-bacterial interactions and pilIX mutants, which are unable to form aggregates, are unable to multiply at the cell surface. The inter-bacterial interactions generated by the Tfp are therefore required for bacteria to form microcolonies at the cell surface. Bacterial spreading onto the host cells relies also on the ability of bacteria to retract their pili. Indeed, following the interactions between bacteria and target cells, in some systems, pili have been shown to retract and eventually adherent meningococci appear non-piliated. Measurements using optical tweezers showed that retraction of a single Tfp generates forces up to 110 pN, in a transient manner for each fiber. Bundles of Tfp, which result from the association of 8 to 10 pili, act as coordinated retractable units. Bundles can generate retraction forces in the nanonewton range. The successive extension, binding and retraction of Tfp enable bacteria to move by twitching motility and spread on the apical surface of the host cells. Furthermore, it has been recently shown that the addition of a phosphoglycerol on pilin, which is increased during bacterial cell interaction, enhances the ability of the bacteria to detach from the adherent aggregates and to disseminate.

Consequences of the Type IV Pilus-Mediated Interaction with Brain Endothelial Cells

As mentioned above, bacterial adhesion onto endothelial cells is mediated by the type IV pili that are responsible for the bacterial-cell and bacteria-bacteria interactions leading to the formation of the microcolonies. Following bacterial adhesion on the apical surface of the host cells, N. meningitidis induces elongation of microvilli toward the bacteria. Interestingly, the formation of such protrusions was also observed in vivo onto brain endothelial cells by transmission electron microscopy analysis of brain sections from a child who died from fulminant meningitis. These cellular projections are likely to be required to allow the microcolonies to stand up to the shear stress of the bloodstream. These observations strongly suggest that such morphological modifications of the host cell membrane are essential in meningococcal pathogenesis.

N. meningitidis induces the clustering of cellular receptors. Following Tfp-mediated adhesion, N. meningitidis induces at the site of bacterial cell interaction the formation of a specific membrane domain enriched in structural proteins and membrane receptors designated the "cortical plaque." This structure dramatically modifies the molecular equilibrium of the host cell, and leads to the opening of the blood-brain barrier (Fig. 1). The formation of such structure is a two steps process: (1) the initial recruitment of one or several receptors following pilus-mediated adhesion and (2) the activation of one or several of these receptors responsible for the recruitment of other transmembrane components and for the modification of the host cell cytoskeleton. This latter step is also mediated by the type IV pili (Fig. 1).

The CD46 receptor was first proposed as being the adhesion receptor for both N. gonorrhoeae and N. meningitidis. However, this finding was not confirmed by subsequent studies. The Laminin receptor was also described as a potential receptor for N. meningitidis. Two bacterial ligands for this receptor have been
reported, the PilQ secretin and the PorA protein. However, considering that PilQ is expressed in PilE non-piliated mutant and that non-piliated non-capsulated strain are unable to interact with endothelial cells in flow conditions, its role remain to be précised. It should be pointed out that the I-domain-containing integrins were described to be essential for Neisseria gonorrhoeae adhesion to epithelial cells. However, similar data have not been reported for N. meningitidis and endothelial cells.

The initial cellular component of brain endothelial cell that interacts with the type IV pili remains unknown. On the other hand, among one of the receptors recruited following meningococcal initial adhesion, the β2-adrenergic receptor was recently described as an important signaling receptor for N. meningitidis. The β2-adrenergic receptor is a G protein coupled receptor (GPCR) that interacts with β-arrestins and the heterotrimeric Gαs protein. This receptor is also known for its implication in vascular homeostasis and disease. The expression of both the β2-adrenergic receptor and β-arrestins is sufficient to promote a N. meningitidis induced cell response in an incompetent cell line such as HEK293. It has been shown that PilE and PilV, the major pilin subunit and a minor pilin subunit, respectively, directly interact with the extracellular N-terminal domain of the β2-adrenergic receptor to transmit the signal.

This interaction is believed to modify the conformation of the receptor, resulting in the activation of the β-arrestins pathway without activating the heterotrimeric Gαs protein and its downstream adenyl cyclase/cAMP pathway, a property referred to as biased activation. β-arrestins are scaffolding proteins involved in many cellular processes such as receptor internalization and actin polymerization. Following the activation of the β2-adrenergic receptor by N. meningitidis and the accumulation of β-arrestins, the signal leads to the formation of a “raft-like” membrane domain enriched in cholesterol and PIP2 in which several transmembrane receptors and structural proteins are sequestered, thus leading to the subsequent formation of the cortical plaque. It is likely that accumulated β-arrestins underneath bacteria plays a major role in the sequestration of these signaling molecules.

The interaction domain between the bacterial colony and the host cell can be compared with a synapse since (1) it is composed of adhesion receptors and signaling receptors (that are mostly immunoglobulin domain containing receptors and G protein coupled receptors, respectively) and (2) it transmits signals. One particularity of this “bacterial synapse” is that adhesion receptor and signaling receptor are not internalized but sequestered underneath the meningococcus (Doulet et al. and personal observation). This “bacterial synapse” is maintained by a cortical network highly enriched in members of the ezrin-radixin-moesin (ERM) proteins family that are anchored to the plasma membrane by their PIP2 binding domain and control the organization of the cortical actin cytoskeleton through their C-terminal F-actin binding sites. ERM proteins also bind the cytoplasmic domain of several ERM binding transmembrane receptors such as CD44 and ICAM-1 through their C-terminal domain and act as linkers between the actin cytoskeleton and the plasma membrane. As a consequence of the recruitment of ERM proteins, many receptors known to be involved in leukocytes adhesion are sequestered underneath bacterial colonies. During leukocytes adhesion, these components that form the “endothelial docking structures” or “transmigratory
cups” are essential to promote firm adhesion and extravasation of leukocytes through the endothelium. Transmigratory cups result from the dynamic redistribution of ICAM-1, VCAM-1, E-selectin and CD44 at the endothelial-leukocyte contact area, accompanied by the recruitment of activated ERM proteins, and leads to cortical actin polymerization.96-98 Since the same set of endothelial proteins is present within the membrane protrusions induced by *N. meningitidis* and within the docking structures promoted by leukocyte adhesion, it was suggested that *N. meningitidis* hijacks the leukocyte adhesion pathway.91

In summary, following the initial adhesion of the bacteria to a yet unknown receptor, the β2-adrenergic receptor is recruited and activated by components of the type IV pili, thus inducing the formation of the cortical plaque.

**Consequences of *N. meningitidis* induced signaling.** The formation of the cortical plaque is accompanied by a robust actin polymerization leading to the elongation of membrane protrusions around bacteria that, as mentioned above, are likely to play a role in the resistance of the colony to flow shear stresses.92 Actin polymerization relies on the activation of the small GTPases of the Rho family.93,94 In addition, it has been demonstrated that proper actin polymerization in these membrane protrusions relies on cortactin phosphorylation. Cortactin or cortical actin binding protein is a perinuclear cytoplasmic protein that is involved in the reorganization of the cell cortical actin cytoskeleton. It appears that cortactin recruitment and phosphorylation is finely tuned by *N. meningitidis* (Fig. 2):

1. The recruitment of cortactin at the site of *N. meningitidis* adhesion is controlled by the Cdc42-Par6/PKCI pathway.84 This pathway is involved in the proper tethering of microtubules at the leading edge of migrating cells.99,100 In the case of the meningococcal infection, the Cdc42-Par6/PKCI pathway may tether microtubules to the site of bacterial adhesion, thus allowing p120-catenin dependent traffic of the cortactin along the microtubules network.84,101,102

2. The activation of cortactin through phosphorylation is controlled by the tyrosine kinase Src that is itself sequestered and activated in the cortical plaque by direct interaction with the β-arrestins.85,103

3. Finally, the ErbB2 tyrosine kinase receptor regulates Src activity and the subsequent cortactin phosphorylation.104 The ErbB2 tyrosine kinase receptor belongs to the family of epidermal growth factor (EGF) receptors. The interaction of *N. meningitidis* with human endothelial cells leads to its activation most likely via formation of ErbB2 homodimers in the cortical plaque. This is an example of a secondary signaling activated by the accumulation of...
a cellular receptor at site of bacterial adhesion that contributes to the formation of the cortical plaque.

Another consequence of the formation of the cortical plaque is the opening of the interendothelial junctions allowing the transmigration of bacteria by a paracellular route. Indeed, it has been shown that \textit{N. meningitidis} recruits junctional proteins underneath the colony into the cortical plaque. This recruitment is due to the activation of the Cdc42-Par3/Par6/PKC\_\zeta pathway that is usually involved in the formation of adherens and tight junctions at cell-cell contact. Here, the ectopic activation of the polarity complex Par3/Par6/PKC\_\zeta leads to abnormal recruitment of junctional proteins that are sequestered underneath bacterial colonies through their interaction with \(\beta\)-arrestins. As a consequence, these molecules are depleted at the intercellular junctions causing endothelium leakage. Adhesion of \textit{N. meningitidis} promotes the cleavage of occludin (a component of the tight junction) by the metalloprotease MMP-8,\(^{105}\) further altering the intercellular junctions.

**Conclusion**

Recent exciting findings have considerably expanded our understanding of the cellular events involved in meningooccal interaction with brain endothelial cells, thus leading to the opening of the paracellular route and meningeval invasion. However, in spite of recent advances in our understanding of these molecular mechanisms, much remains to be discovered about the complex molecular networks involved. Among major issues is the identification of the receptor for meningooccal adhesion, which would constitute a significant breakthrough in the field and the identification of animal models that would allow us to confirm these in vitro observations.

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Virulence

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