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Defense at the border: the blood–brain barrier versus bacterial foreigners

Nina M van Sorge[1,2] and **Kelly S Doran**[*,2,3]

[1]University Medical Center Utrecht, Medical Microbiology, Heidelberglaan 100, G04.614, 3584 GX Utrecht, The Netherlands [2]Department of Pediatrics, University of California San Diego, 9500 Gilman Drive, CMME 1088, La Jolla, CA 92093-0687, USA [3]Department of Biology & Center for Microbial Sciences, 5500 Campanile Dr. San Diego State University, San Diego, CA 92182, USA

Abstract

Bacterial meningitis is among the top ten causes of infectious disease-related deaths worldwide, with up to half of the survivors left with permanent neurological sequelae. The blood–brain barrier (BBB), composed mainly of specialized brain microvascular endothelial cells, maintains biochemical homeostasis in the CNS by regulating the passage of nutrients, molecules and cells from the blood to the brain. Despite its highly restrictive nature, certain bacterial pathogens are able to gain entry into the CNS resulting in serious disease. In recent years, important advances have been made in understanding the molecular and cellular events that are involved in the development of bacterial meningitis. In this review, we summarize the progress made in elucidating the molecular mechanisms of bacterial BBB-crossing, highlighting common themes of host–pathogen interaction, and the potential role of the BBB in innate defense during infection.

Keywords

blood–brain barrier; *Escherichia coli* K1; group B *Streptococcus*; *Haemophilus influenzae* type B; meningitis; *Neisseria meningitidis*; neutrophil; *Streptococcus pneumoniae*; tight/adherens junction

Dynamic epidemiology of bacterial meningitis

Despite our enormous progress in the treatment and prevention of infectious diseases, there are still 1.2 million cases of bacterial meningitis per year, 170,000 of which are fatal [201]. In addition, permanent neurological sequelae occur in up to 50% of survivors [1,2]. Although many bacteria can cause disease in humans, only a limited number of pathogens are isolated from the CNS of patients. The most prevalent cause of meningitis varies depending on geographic location, socioeconomic status, age, vaccination availability and overall health status of the individual.

Globally, *Streptococcus pneumoniae, Neisseria meningitidis* (meningococcus) and *Haemophilus influenzae* type B (HiB) are the most common causes of meningitis in infants

[*]Author for correspondence: Tel.: +1 619 594 1867 ■ Fax: +1 619 594 5676 ■ kdoran@sciences.sdsu.edu.

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and adults. In industrialized countries, both the incidence and epidemiology of meningitis have been dramatically affected by the introduction of vaccines against these organisms. Before the introduction of the conjugate HiB vaccine in the 1980s, HiB alone accounted for approximately half of the cases of meningitis in infants, compared with a 78% reduction in disease incidence following routine vaccination [3,4]. Globally, however, HiB is estimated to still cause 173,000 cases of meningitis each year [5] as HiB vaccination programs are not yet available in developing countries [4]. Strategies are in place to introduce the HiB vaccine in resource-poor countries over the next several years [202]. Currently, *S. pneumoniae* is the worldwide leading cause of bacterial invasive disease in children younger than 5 years of age [6] and of bacterial meningitis in all age groups except young infants [7]. In addition, in sub-Saharan Africa, also called the 'meningitis belt', *N. meningitidis* is a leading cause of large epidemics of meningococcal meningitis. Vaccination strategies against *S. pneumonia* and *N. meningitidis* have been limited to several serotypes and are overall hampered by concurrent increases in nonvaccine serotypes [8-10]. Therefore, a complete elimination or prevention of pneumococcal and meningococcal meningitis will only be possible when vaccines either include all serotypes, or a conserved antigen present in all disease isolates.

Although not classically thought of as a meningeal pathogen, non-typhoidal *Salmonella* (NTS) species are a frequent cause of meningitis in certain parts of Africa [11-13]. In northern Uganda, it has even been reported as the second most common cause of pediatric meningitis after the introduction of the HiB vaccine in 2002 [14]. The incidence of NTS meningitis is likely to increase given that NTS has become the most common cause of bacteremia in tropical Africa [15]. NTS meningitis has a high case fatality rate (up to 60%) and a high incidence of postinfectious sequelae in survivors as treatment is often complicated by multidrug resistance [11-13,15,16].

A different pathogen spectrum is associated with meningitis in the newborn. In many industrialized countries, *Streptococcus agalactiae*, also known as group B *Streptococcus* (GBS), *Listeria monocytogenes* and *Escherichia coli* K1 are the most common causes of meningitis in the neonatal period [7,17-19]. By contrast, in developing countries, additional pathogens such as *Klebsiella, Staphylococcus aureus*, and NTS species, are frequently isolated from neonates, and GBS is usually less common compared with many industrialized countries [20,21]. No vaccination strategies are currently in place to prevent these types of infections but they are anticipated to reduce the number of meningitis cases [7]. Fortunately, increased screening and intrapartum antibiotic prophylaxis has resulted in a decline in early-onset GBS invasive disease in the USA [22,23]. However, this treatment has not eliminated the incidence of GBS meningitis and concern has been raised about coinciding increases in non-GBS early-onset invasive disease, especially in preterm infants as a result of increased antibiotic use [24,25].

Besides these frequent causes of meningitis in different age groups, additional pathogens are known to cause meningitis in vulnerable groups, such as immunocompromised patients, which includes infants and the elderly adults (>65 years of age), or specific population groups. Meningitis due to *Mycobacterium tuberculosis* and NTS species occurs relatively frequently in HIV-infected and other immunocompromised patients [26-28], whereas *Streptococcus suis* is the most common cause of acute bacterial meningitis in southeast Asia, but is rare in other countries [29,30]. Although still considered a rare complication, increasing numbers of *S. aureus* meningitis cases are observed in the hospital setting [31], usually as a result of postoperative complications or hematogenous spread due to underlying diseases such as HIV, immune deficiencies, diabetes or cardiovascular disease [31,32]. The mortality rate seems especially high when disease was contracted in the community [31-34]. Finally, *Bacillus anthracis*, an exotic cause of infection and meningitis overall, has gained research interest as a result of its use in bioterrorism attacks in 2001 [35]. In addition to its

potential use as a bioweapon, *B. anthracis* is still a relevant natural cause of infection to people living in endemic regions in agricultural settings or exposure due to occupation [36]. Just recently, an outbreak of systemic anthrax occurred in Scotland among intravenous drug users due to injection of contaminated heroin [37]. Anthrax meningitis is the main neurological complication of systemic infection, approaching 100% mortality despite intensive antibiotic therapy [38].

The pathogenesis & pathophysiology of bacterial meningitis

Many of the meningeal pathogens, including HiB, *N. meningitidis, S. pneumoniae*, GBS and *S. aureus*, are able to colonize the skin, upper respiratory, nasopharyngeal, GI or vaginal tract of healthy individuals. Carriage can be stable, transient or intermittent [39] and often remains asymptomatic. However, in certain cases, bacteria penetrate host cellular barriers to initiate a local infection that can result in systemic spread. An association between high-level bacteremia and development of meningitis has been suggested for *E. coli, S. pneumoniae*, GBS and HiB from experimental models of hematogeneous meningitis [40-45]. This implies that bloodstream survival is an important virulence trait of meningeal pathogens to avoid immune clearance by complement- and antibody-mediated phagocytic killing by host immune cells. Indeed, once bacteria reach the bloodstream, their transcriptional profile changes dramatically and includes survival strategies such as an altered cell membrane or cell wall composition [46,47] and increased expression of complement regulatory proteins [48,49] or iron-uptake systems [48]. Another strategy to prevent bloodstream clearance is adopted by *E. coli* K1 through expression of OmpA. This bacterium initiates its specific uptake in macrophages and dendritic cells through FcγRIa (CD64), and in neutrophils through gp96, to replicate intra-cellularly [50]. In this niche, the bacterium is protected from serum bactericidal activity and from phagocyte-mediated clearance by suppressing oxidative burst [51-53]. Increasing bacterial clearance from the blood would prevent or limit bacterial survival and multiplication, thereby limiting the possibility to reach the CNS. Vaccination poses a successful strategy to limit the risk for developing meningitis as it significantly decreases carriage, and reduces the levels of bacteremia due to enhanced phagocytic clearance by neutrophils and macrophages for vaccine-included serotypes. Once disease is established, early and aggressive antibiotic therapy can help reduce the bacterial load associated with disease progression.

Following bloodstream survival, bacteria will ultimately leave the bloodstream and invade the CNS, resulting in inflammation of the meninges, increased BBB permeability and pleocytosis. The molecular mechanisms involved in bacterial penetration of the BBB are complex, and common themes are described in more detail below. Subsequent CNS tissue injury results from cerebral ischemia, edema, hydrocephalus and increased intracranial pressure [54] and is caused by both toxic bacterial products and host inflammatory pathways initiated to clear the infection. In particular, the excessive inflammatory response of neutrophils has been associated with increased CNS injury [55,56]. Consequently, several large clinical trials have studied the potential benefit of corticosteroid therapy to improve disease outcome by reducing unwanted inflammatory responses [57-60]. Overall, adjuvant corticosteroid therapy does not decrease mortality [61], although it does reduce hearing loss and neurological sequelae, but only in high income countries [61].

BBB composition

The BBB is a structural and functional barrier that maintains the homeostasis of the neutral microenvironment by impeding the passage of virtually all molecules except those that are small and lipophilic [62,63]. The BBB is composed of brain microvascular endothelial cells (BMECs) that line cerebral microvessels along with periendothelial structures, which

include pericytes, astrocytes and a basal membrane [64]. Together, these cells function in controlling the infiltration of blood proteins and cells through the vessel wall and into underlying tissues and in general provide functional barrier properties [65]. Brain endothelial cells are distinguished from the other cells of the BBB by possessing fewer cytoplasmic vesicles, more mitochondria and a large number of intercellular junctions that promote high transendothelial electrical resistance and retard paracellular flux [66]. Similar to epithelial cells, endothelial cells possess adherens junctions (AJs) and tight junctions (TJs) at their intercellular contacts [67,68]. Although TJs and AJs are formed by different molecules, cell–cell adhesion is accomplished similarly by a trans-membrane protein that interacts with the actin cytoskeleton and/or signaling proteins through an adaptor protein [65]. AJs in BMECs are dependent on the interaction between the cytoplasmic tail of cadherins with catenins, which in turn are linked to the actin cytoskeleton and/or signaling components [69]. TJs in BMECs are composed of four integral membrane proteins (occludin, claudins, junctional adhesion molecules and cell-selective adhesion molecules) that are linked through cytoplasmic proteins (ZO-1, -2 and -3 and cingulin) to the actin cytoskeleton [68,70,71]. Numerous endothelial functions are mediated by these junctional structures including maintenance of cell polarity, signaling, modulation of transcription and endothelial stabilization [72-74]. Another critically important function of AJs and TJs is regulation of BBB permeability. Until recently, AJs were considered to be important for basic cell–cell adhesion but not critically involved in regulating BBB permeability. However, a recent study demonstrated cross-talk between the TJ and AJ. Homophilic interactions between vascular endothelial cadherin expressed in AJs were demonstrated to regulate expression of TJ claudin-5 [75], providing a molecular mechanism for increased permeability when interfering with AJ formation.

Transcellular penetration of the BBB

The initial attachment of blood-borne bacteria to brain endothelium and subsequent invasion may represent the initial step in penetration and/or disruption of the BBB. This interaction involves a complex interplay between host receptors and bacterial components. In recent years, significant progress has been made in understanding the molecular interaction between the BBB and meningeal pathogens because of the availability of *in vitro* tissue culture models of human BMECs (hBMECs) [76-78] and *in vivo* animal models of hematogeneous meningitis [79-83]. Many meningeal bacteria cross the BBB transcellularly as live organisms, requiring host actin cytoskeletal rearrangements that promote the initial bacterial uptake into the cell [78,84-91]. The signal transduction pathways involved display some common themes shared by different organisms, as will be discussed in more detail below. Most *in vitro* work has focused on identifying the bacterial molecules and host receptors involved in bacterial adherence to, and invasion of, the BBB, as recently reviewed by Kim [92,93]. Identification of critical bacterial adhesion/invasion molecules can be achieved using different approaches. An unbiased and comprehensive approach to identify new bacterial components involved in BBB interaction uses random mutant libraries and screens for loss of adherence and/or invasion [94,95]. This approach identified lipoteichoic acid as a critical mediator in brain endothelial cell invasion for GBS, *S. aureus* and *S. pneumoniae* both *in vitro* and *in vivo* [83,95]. Complementarily, one can determine critical adhesins and invasins by analyzing bacterial transcriptional profiles [87,96,97] or differential fluorescence gene induction [98] during BBB interaction. Subsequent ana lysis of hBMEC adherence/invasion using defined isogenic mutants of candidate genes is needed to confirm protein involvement. For example, this approach identified a strong contribution for type I fimbriae in *E. coli* K1 in hBMEC adherence [97]. Bioinformatic approaches comparing bacterial whole genome sequences can also identify key molecules as recently exemplified for the HvgA protein found in hypervirulent GBS strains [99]. In addition to expression of specific adhesion molecules, brain tropism seems to be critically determined

by low blood flow in brain capillaries, as was recently demonstrated for *N. meningitidis* [100]. Importantly, bacterial adherence to endothelial cells was similar under low flow versus static conditions [100].

From the body of work studying molecular interactions between the BBB and bacteria, some commonalities in the way different Gram-positive and Gram-negative bacteria interact with brain endothelial cells can be discerned. First, many meningeal bacteria, including *E. coli* K1 [97], GBS [101,102] and *N. meningitidis* [103] use pili (also called fimbriae) or the shorter version, fibrils [104], to initiate binding to hBMECs. A similar mechanism is probably involved in BMEC interaction with *S. pneumoniae* [105,106] and HiB [107,108] as their pili or fibrils have been shown to contribute to interaction with other epithelial and endothelial cell types. Second, many meningeal pathogens possess a capsular polysaccharide [80,84,109-111], which promotes bloodstream survival and high-level bacteremia prior to BBB penetration. Studies performed using *in vitro* assays demonstrate that the capsule actually inhibits bacterial invasion, probably due to electrostatic repulsion or masking of bacterial surface structures that could function as adhesins. Thus, it is likely that capsule expression is highly regulated and may be induced during bloodstream replication and repressed while on mucosal or endothelial cell surfaces [86,112]. Finally, the expression of bacterial toxins can increase BBB penetration through different mechanisms. In the case of *E. coli* K1, cytotoxic necrotizing factor-1 activates RhoA, resulting in increased invasion of brain endothelial cells *in vitro* and increased penetration of the BBB *in vivo* [113]. By contrast, GBS β hemolysin (β-h/c) [84], *S. pneumoniae* pneumolysin [114] and HiB lipopolysaccharide [115] damage brain endothelial cells, resulting in increased BBB permeability *in vivo* [80,116].

A broad range of host receptors has been described to mediate interaction with meningeal pathogens (reviewed by [92]); also here, common themes can be recognized. First, bacterial binding to host receptors on brain endothelium seems to be important for initial attachment. The laminin receptor (LR) and platelet activating factor receptor (PAFr) have been identified as common portals of CNS entry for the leading meningeal pathogens *N. meningitidis, S. pneumoniae* and *H. influenzae* [86,117-119]. In addition, LR was identified in previous studies as an important internalization receptor for *E. coli* K1 on hBMECs [120,121]. Different bacterial adhesins, *N. meningitidis* PilQ and PorA, *S. pneumoniae* CbpA and *H. influenzae* OmpP2, all target a common carboxy-terminal domain of LR to establish initial contact with brain endothelium [117]. Sequential binding to PAFr through bacterial surface phosphorylcholine results in β-arrestin-mediated pneumococcal invasion of brain endothelial cells [86,122], and interfering with PAFr-mediated uptake protects the host from pneumococcal meningitis *in vivo* [122,123]. It is likely that both *N. meningitidis* and *Haemophilus* species similarly interact with PAFr on brain endothelial cells as both express phosphorylcholine on their pili and lipopolysaccharide, respectively [119,124-126]; however, further experimentation is needed to definitively demonstrate this. Second, the BBB interaction is not always direct but may involve bridging molecules such as components of the extracellular matrix. For example, human collagen bridges GBS pili adhesin, PilA, and α2β1 integrin on BMECs resulting in bacterial attachment, immune activation and ultimately penetration of the CNS [56]. Similarly, fibronectin bridges *N. meningitidis* and the α5β1 integrin on BMECs, promoting bacterial internalization [109]. Also, glucosaminoglycans on brain endothelium, which are also known to interact with integrins [127], have recently been shown to bind the αC protein of GBS [128]. Therefore, the LR, PAFr and integrin signaling pathways may represent attractive targets for therapeutic intervention, as they would prevent a broad range of bacterial meningitis. Third, inflammatory activation of brain endothelial cells by cytokines, which are typically elevated in meningitis patients [129-134], can increase host receptor expression resulting in enhanced bacterial invasion. For example, stimulation of cells with TNF-α, IL-1 or TGF-β resulted in

increased bacterial uptake [86,135-137]. Some bacteria take advantage of this mechanism to promote their own uptake; the surface-expressed neuraminidase, NanA on *S. pneumoniae* promotes penetration of the BBB by inducing chemokine release from brain endothelial cells [82,136]. Ultimately, a more detailed understanding of host–bacteria interactions at the molecular level may result in therapeutic treatment strategies to block bacterial entry at an early stage of infection.

Breakdown of BBB integrity

Besides evidence of transcellular migration, many *in vitro* studies have demonstrated that bacteria can affect endothelial barrier integrity, allowing for direct entry or paracellular translocation. As discussed above, BMECs are tightly interconnected by AJs and TJs, which limit paracellular passage of foreign particles including bacteria. Disruption of the BBB is a hallmark event in the pathophysiology of bacterial meningitis. Many meningeal pathogens affect endothelium barrier integrity by direct toxic effects and/or by interfering specifically with AJ/TJ formation. For example, GBS and *S. pneumoniae* directly affect barrier function by secreting a pore-forming toxin [84,114,138]. For GBS, higher toxin production has been associated with an increased capacity to cause meningitis [80]. In addition to pathogen-derived toxins, increased expression of inflammatory cytokines/chemokines/molecules by the host in response to infection can negatively impact BBB function and disease outcome. Increased systemic expression of TNF-α is linked to enhanced permeability of the BBB [139-141]. In addition, *E. coli* K1 (through OmpA), *S. pneumoniae* and GBS increase nitric oxide production from brain endothelial cells by inducing expression of inducible nitric oxide synthase (iNOS) [142-145]. Consequently, this attenuates BBB integrity [142,143] and promotes bacterial invasion in the case of *E. coli* K1 [142]. However, inhibition of iNOS function does not provide unambiguous answers; in the case of GBS meningitis, pharmacological inhibition of iNOS results in increased pathology [144], whereas genetic deletion of iNOS confers complete protection in models of *E. coli* K1 meningitis [146].

Besides general BBB insult, some pathogens use sophisticated strategies to target endothelial cell junctions to promote barrier permeability resulting in increased bacterial BBB traversal. The critical AJ protein, vascular endothelial cadherin, is a common target for meningeal pathogens. *E. coli* K1 and *N. meningitidis* use their surface adhesion molecules to exploit brain endothelial cell signaling to increase paracellular translocation [147-150]. OmpA-expressing *E. coli* K1 causes permeability changes by dissociating β-catenins from cadherins and activating protein kinase Cα [150]. For meningococcal meningitis initial observations suggested that attachment of meningococci to brain endothelial cells through type IV pili signals the formation of mislocalized AJs, opening up the paracellular route for *N. meningitidis* translocation into the CNS [148]. In a follow-up study, the β2-adrenoceptor/β-arrestin signaling pathway was found to be involved in the induced junctional protein rearrangements and bacterial crossing of the BBB [147]. In addition to targeting AJs, *N. meningitidis* induces specific cleavage of the TJ component occludin through the release of host matrix metalloproteinase 8, resulting in endothelial cell detachment and increased paracellular permeability [149]. Pharmacological intervention of OmpA–hBMEC and type IV pili–β2-adrenoceptor interaction would therefore not only block the initial interaction of bacteria with the BBB, but would also prevent exploitation of host signaling machinery by the pathogen.

In contrast to *E. coli* K1 and *N. meningitidis*, which use surface-associated molecules to affect BBB permeability, *B. anthracis* disrupts both AJs and TJs by secreted non-pore-forming factors including proteases and edema and lethal toxin complexes. Whereas edema toxin and secreted protease InhA affect the distribution of the critical TJ component ZO-1 [151-153], AJ formation is blocked by a synergistic effect of lethal toxin and edema toxin on

endosomal transport of cadherins [154]. Disruption of endosome recycling by anthrax toxins has more widespread effects on vascular function as important cell-cell communication pathways, such as the Notch signaling pathway, also depend on proper endosome transport [154].

Finally, host inflammatory factors may also contribute to break down of the BBB. It was recently demonstrated that host neutrophils contribute to BBB permeability during the pathogenesis of GBS meningitis [56]. Although neutrophils were found to be critically important to prevent bacterial sepsis, depletion of peripheral neutrophils in a murine hematogenous model of GBS meningitis prolonged survival, and decreased bacterial brain load and BBB permeability in GBS-infected mice [56]. Correspondingly, prevention of leukocyte infiltration into the CNS using anti-CD18 antibody was previously shown to improve pneumococcal and HiB meningitis outcome [155,156].

The BBB innate defense response

Many studies have shown that the host's inflammatory response contributes to many adverse events during bacterial meningitis. However, little is known about the role of the BBB as a functional, rather than just a physical, barrier against the initial threat of an invading pathogen. A better understanding of the host BBB responses to pathogen infection can aid in the development of preventive therapies for CNS infection.

The first comprehensive microarray ana lysis of the BBB transcriptional response to a pathogen was examined during GBS infection [80]. Interestingly, in this and subsequent studies, infection triggered a specific gene expression program for neutrophil recruitment (i.e., IL-8, CXCL1, CXCL2, ICAM-1) and activation (IL-6, IL-8), which was strongly dependent on GBS β-h/c toxin [80], the transcriptional regulator of virulence, CovR [138] and PilA expression [56]. Strong proinflammatory cytokines such as TNF-α or IL-1 were absent [80], precluding the development of an unchecked pattern of activation that is detrimental to CNS structures. Subsequent comparative microarray experiments with *S. pneumoniae*, HiB and *Salmonella enterica* Typhimurium reveal the presence of a core hBMEC transcriptional response in addition to pathogen-specific gene expression profiles [van sorge NM, doran KS, Unpublished Data] [89,136]. A group of core response genes function to orchestrate neutrophil recruitment (IL8, CXCL1, CXCL2), extravasation (ICAM-1), activation (IL6, IL8) and survival (GM-CSF). Similarly, other meningeal bacteria including *E. coli* K1 [157], *S. suis* [158], *L. monocytogenes* [159] and *N. meningitidis* [160,161] have been reported to induce the upregulation and secretion of neutrophil-specific factors upon hBMEC infection. Again TNF-α and IL-1 are typically absent from this response, except during infection with HiB [van sorge NM, doran KS, Unpublished Data] or *N. meningitidis* [161]. Whether this broader spectrum of inflammation in response to HiB or *N. meningitidis* contributes to the high prevalence of these two pathogens in bacterial meningitis or the often fulminant disease course during infection, remains to be determined. Overall, these data suggest that the BBB response serves a sentinel function by recognizing the threat of a bacterial pathogen, resulting in effective clearance of the bacteria before it can enter the CNS. However, the timing and magnitude of the neutrophil recruitment response is critical for the outcome of infection. Continued exposure and invasion of the pathogen may result in overactivation of BBB endothelium leading to increased inflammation that may compromise BBB integrity or cause neuronal damage. Indeed, experimental delay of neutrophil apoptosis results in prolonged inflammatory activity and more severe disease in a mouse pneumococcal meningitis model [55]. A similar observation was recently published for GBS meningitis. Pilus adhesion protein PilA, located at the tip of the pilus structure [162], induces hBMEC IL-8 expression through α2β1 integrin/focal adhesion kinase signaling [56]. In an *in vivo* model of GBS

meningitis, PilA-induced host signaling resulted in a disproportionate neutrophil inflammatory response that increased BBB permeability, bacterial CNS penetration and mortality [56]. In the case of anthrax meningitis, the opposite has been observed. Infection of hBMEC with avirulent *B. anthracis* initiates the common neutrophil recruitment response as observed with other meningeal pathogens. However, the presence of the toxin-encoding pXO1 plasmid results in active downregulation of this innate defense pathway [163]. The resulting suppression of neutrophil chemotaxis allows for unrestricted proliferation and dissemination of the bacteria into the CNS [163].

Analysis of changes in transcriptional regulation upon BBB infection is just a starting point for future studies. It will be important to use this information to determine the signal transduction pathways and initiating receptors involved in this response, and whether their activation contributes to host resistance or neuronal damage. Based on published studies, some common downstream signaling pathways for bacterial internalization into hBMECs can be identified. Both *E. coli* K1 and GBS activate focal adhesion kinase signaling to trigger internalization in hBMECs [56,164,165]. Similarly, *S. pneumoniae* and *N. meningitidis* both trigger β-arrestin signaling downstream of PAFr and β2-adrenoceptor, respectively [147,148], which ultimately results in bacterial translocation across the BBB. Interestingly, the effects downstream of β-arrestin are different in both cases: *S. pneunoniae* β-arrestin signaling triggers vacuolar trafficking across brain endothelial cells [122], whereas *N. meningitidis* β-arrestin signaling depletes intercellular junctions, allowing paracellular translocation across the BBB [147].

In addition to signaling involved in bacterial translocation, unraveling signaling pathways that trigger protective or unwanted cytokine release could aid the development of pharmacological intervention strategies. For example, *N. meningitidis,* GBS and *S. pneumoniae* trigger IL-6 and IL-8 production in hBMECs by activating MAPK pathways [56,136,160]. The upstream receptors that induce this MAPK signaling have not been identified but probably involve immune pattern recognition receptors, such as integrins, Toll-like receptors and intracellular NOD-like receptors. Interestingly, in all these studies it was found that activation of cytokine responses did not require bacterial invasion of brain endothelial cells [56,136,160], suggesting that signaling is initiated at the host cell surface. Also, for other bacteria, this dissociation between bacteria-host cell interaction and cytokine production has been observed [157,166,167]. This suggests that interference with bacterial attachment to brain endothelium could prevent many of the downstream effects of meningitis pathogenesis.

Future perspective

Significant progress has been made in identifying molecular mechanisms that contribute to bacterial-BBB interaction and signaling during the progression of CNS disease. Identification of common pathways employed by bacterial pathogens to cross and penetrate BBB endothelium will assist in the identification of important bacterial and host cell targets for the development of effective therapies. However, a multi-disciplinary and systems biology approach is necessary to incorporate all this knowledge into new testable hypotheses that will provide insight into the pathogenesis and pathophysiology of bacterial meningitis and the discovery of novel therapeutic strategies.

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Executive summary

Dynamic epidemiology of bacterial meningitis

- There are still 1.2 million cases of bacterial meningitis per year, 170,000 of which are fatal and survivors are left with permanent neurological sequelae.
- Only a limited number of bacterial pathogens are capable of causing meningitis, the severity of which varies depending on geographic location, socioeconomic status, age, vaccination availability and overall health status of the individual.

The pathogenesis & pathophysiology of bacterial meningitis

- In order to cause meningitis, bacterial pathogens must survive in the bloodstream and penetrate or transmigrate across the blood–brain barrier (BBB), which is primarily comprised of a single layer of specialized endothelial cells.
- Once inside the CNS, the bacteria multiply and induce inflammation of the subarachnoid and ventrical spaces with associated pathophysiologic alterations such as increased BBB permeability and pleocytosis.

BBB composition

- The BBB is composed of a single cell layer of brain microvascular endothelial cells that line cerebral microvessels.
- Brain endothelial cells contain adherens and tight junctions that act to impede the passage of virtually all molecules, thereby maintaining the microenvironment of the CNS.

Transcellular penetration of the BBB

- The attachment of blood-borne bacteria to brain endothelium and subsequent invasion may represent the initial step in penetration and/or disruption of the BBB; this interaction involves a complex interplay between host receptors and bacterial components.

Breakdown of BBB integrity

- Many meningeal pathogens are capable of disrupting endothelium junction complexes by direct toxic effects, interfering specifically with junctional formation, and/or induction of an inflammatory response, which itself may compromise BBB integrity.

BBB innate defense response

- Studies suggest that the BBB responds to bacterial encounter with a core gene activation program orchestrated to promote the targeted recruitment and activation of neutrophils.
- Continued exposure and invasion of the pathogen may result in overactivation of BBB endothelium, leading to increased inflammation and BBB breakdown.