Host–pathogen interactions in bacterial meningitis

Kelly S. Doran¹² · Marcus Fulde³⁴ · Nina Gratz⁵ · Brandon J. Kim¹ · Roland Nau⁶⁷ · Nemani Prasadarao⁶ · Alexandra Schubert-Unkmeir⁹ · Elaine I. Tuomanen⁵ · Peter Valentin-Weigand³

Received: 10 August 2015 / Revised: 21 December 2015 / Accepted: 22 December 2015 / Published online: 7 January 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract  Bacterial meningitis is a devastating disease occurring worldwide with up to half of the survivors left with permanent neurological sequelae. Due to intrinsic properties of the meningeal pathogens and the host responses they induce, infection can cause relatively specific lesions and clinical syndromes that result from interference with the function of the affected nervous system tissue. Pathogenesis is based on complex host–pathogen interactions, some of which are specific for certain bacteria, whereas others are shared among different pathogens. In this review, we summarize the recent progress made in understanding the molecular and cellular events involved in these interactions. We focus on selected major pathogens, Streptococcus pneumoniae, S. agalactiae (Group B Streptococcus), Neisseria meningitidis, and Escherichia coli K1, and also include a neglected zoonotic pathogen, Streptococcus suis. These neuroinvasive pathogens represent common themes of host–pathogen interactions, such as colonization and invasion of mucosal barriers, survival in the blood stream, entry into the central nervous system by translocation of the blood–brain and blood–cerebrospinal fluid barrier, and induction of meningeal inflammation, affecting pia mater, the arachnoid and subarachnoid spaces.

Keywords Neuroinfectiology · Bacterial meningitis · Pneumococci · Meningococci · Group B Streptococcus · Streptococcus suis · Escherichia coli K1

Introduction

Bacterial meningitis is a serious threat to global health. Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae type b are most commonly associated with bacterial meningitis in infants and adults [150]. In sub-Saharan Africa, also called the ‘meningitis belt’, N. meningitidis is a leading cause of large epidemics of meningococcal meningitis. Further bacteria that cause meningitis in children and adults include Group B Streptococcus (GBS), Escherichia coli K1, non-typhoidal Salmonella, Klebsiella spp., Staphylococcus aureus, Listeria monocytogenes, Mycobacterium tuberculosis and the neglected porcine zoonotic pathogen Streptococcus suis. Many of the meningeal pathogens are able to colonize the skin and different mucosal surfaces of healthy individuals. 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 some bacteria [83, 108]. This implies that survival in the blood is an important virulence trait of meningeal pathogens. Following bloodstream survival or by spread from infectious foci in the vicinity of the brain (mastoiditis, sinusitis), bacteria will ultimately invade the central nervous system (CNS), resulting in inflammation of the meninges, increased blood–brain barrier (BBB) permeability, cerebrospinal fluid (CSF) pleocytosis, and infiltration of the nervous tissue (Fig. 1). Subsequent CNS tissue injury (Fig. 1) results from apoptotic neuronal injury, cerebral ischemia, edema, hydrocephalus and increased intracranial pressure [96] and is caused by both toxic bacterial products and host inflammatory pathways initiated to clear the infection. In

![Fig. 1 Inflammation and neuronal injury in human bacterial meningitis.](image)

*Fig. 1* Inflammation and neuronal injury in human bacterial meningitis. **a** Strong infiltration of the right lateral ventricle by granulocytes and monocytes in *Neisseria meningitidis* meningitis. The double-strand DNA breaks in the nuclei of apoptotic granulocytes are stained black (in situ tailing counterstained with nuclear fast red, ×10). **b** Macrophage after phagocytosis of apoptotic granulocytes (black, arrowheads) and granulocyte at the beginning of the apoptotic process indicated by partial staining of its nucleus (arrow) (*N. meningitidis* meningitis, in situ tailing counterstained with nuclear fast red, ×100). **c** Thrombosis of two small vessels (arrows) and strong perivascular mainly granulocytic infiltrates in the thalamus, *Streptococcus pneumoniae* meningitis (haematoxylin–eosin, ×20). **d** Apoptosis of granule cells in the dentate gyrus of the hippocampal formation, otogenic bacterial meningitis (in situ tailing counterstained with nuclear fast red, ×40). **e** Diffuse axonal injury, *S. pneumoniae* meningitis (amyloid precursor protein immunohistochemistry, counterstaining with hemalum, ×20). Bars represent 120 μm (a), 12 μm (b), 60 μm (c), 30 μm (d), 60 μm (e).
particular, the excessive inflammatory response of neutrophils (PMNs) has been associated with increased CNS injury [57] (Fig. 1). This review summarizes recent progress made in our understanding of host–pathogen interactions in bacterial meningitis, exemplified by four of the most common pathogens, *S. pneumoniae*, Meningococcus, GBS, and *E. coli* K1, and a rare but neglected pathogen, *S. suis*).

**Common steps and mechanisms in pathogenesis of bacterial meningitis**

Pathogens causing meningitis often colonize mucosal surfaces and show similar patterns of disease progression. Thus, it is plausible that they share common strategies to advance from the mucosa into the blood stream and further into the brain. An overview of main similarities and differences of the pathogens described in following chapters is given in Table 1. Many bacteria bind to extracellular matrix proteins, e.g., laminin, collagen or fibronectin, to facilitate initial attachment preceding invasion. In addition, some bacterial adhesins, e.g., of *N. meningitidis*, also bind to members of the CEACAM family of cell adhesion molecules, others, e.g., OmpA of *E. coli* K1, recognize specific glycoproteins in a lectin-like fashion. Binding of bacterial adhesins to specific host cell receptors may lead to a signal transduction resulting in tight bacterial attachment to or internalization by the host cells. As outlined above (see “*S. pneumoniae meningitis*”) “innate invasion” is a common entry mechanism that counteracts innate immune mechanisms and employs molecular mimicry, as exemplified by PCho mimicking the chemokine PAF. A hallmark of many bacteria infecting the CNS is their ability to survive in the blood stream by either avoiding or protecting against phagocytosis, e.g., by expression of a capsule (*S. suis*) or by entering and persisting in PMNs or macrophages (*E. coli* K1). However, sustained bacteremia is not always a prerequisite for bacterial entrance to the CNS, since meningitis can also be caused by direct invasion from neighboring infected tissues. Nevertheless, all bacteria have to breach certain barriers, such as the BBB and blood–CSF barrier (B-CSFB), to get access to the brain. Translocation across such barriers may occur via a para- or transcellular process, depending on the virulence traits expressed by the pathogen. Cytolytic toxins, e.g., those expressed by *S. pneumoniae*, GBS, *S. suis* and *E. coli*, can damage host cells thereby leading to disruption of the barrier and mediation of paracellular invasion. Transcellular breaching of barriers is based on intracellular invasion, which often involves bacterial exploitation or “hijacking” of signal platforms and pathways, as exemplified by *N. meningitidis*. Once the pathogen has reached the brain, bacteria (or bacterial components) are recognized by resident immune cells, such as microglia and astrocytes, leading to their activation. Furthermore, circulating professional immune cells, such as granulocytes and monocytes/ macrophages, are attracted and subsequently infiltrate the infected brain parenchyma (Fig. 1). Especially in the neonate host, the resulting antibacterial immune response might be overwhelming and not well orchestrated, leading to a pronounced neuronal damage and even death. If the host survives infection, pathogen-specific post-infectious sequelae, such as deafness, blindness or certain kinds of retardation might be the result.

**Streptococcus pneumoniae meningitis**

*Streptococcus pneumoniae*, a Gram-positive extracellular pathogen, is one of the most common etiologic agents of bacterial meningitis worldwide affecting predominantly young children and the elderly. While more commonly a quiescent colonizer of the nasopharynx, this bacterium causes mild infections such as otitis media and sinusitis but also life-threatening conditions such as pneumonia, bacteremia and meningitis. Pneumococcal meningitis is characterized by a high mortality rate (20–30 %) due to complications such as brain edema, cerebral ischemia and increased intracranial pressure arising by an excessive immune response as well as damage by the pathogen itself. Survivors suffer from long-term neurological deficits such as hearing loss and cognitive impairment. Recently discovered reasons for long-term neurological sequelae in pneumococcal meningitis may be focal or diffuse axonal injury (Fig. 1) [87] and synapto- and dendritotoxicity mediated by pneumolysin and glutamate [155].

Most of the findings regarding the pathophysiology of pneumococcal meningitis are either derived from brain autopsies (representing only fatal cases) or from animal models that aim to closely mimic clinical features of human disease. The most prominent models are the mouse, the rabbit and the rat. The use of knockout technology made the mouse a useful model to study the host response to the pneumococcus during meningitis [94]. Also, hippocampal neuronal apoptosis [78] and cortical brain damage have been observed [55] with this model. The rabbit was used to study meningitis-related processes within the CSF, e.g., bacterial growth, antibiotic penetration and components of the immune response [24, 92]. In the rabbit model, apoptotic damage occurs in the dentate gyrus of the hippocampal formation [14, 161]. This form of neuronal injury is present in approx. 70 % of human autopsy cases [88] (Fig. 1d). In the infant rat model, cortical and hippocampal damage have been observed that closely resembles the pattern of necrotic and apoptotic neuronal injury in human pneumococcal meningitis [66,
### Table 1 Main similarities and differences of bacterial pathogens causing meningitis

| Nature of the pathogen | Streptococcus pneumoniae | Neisseria meningitidis | Group B Streptococcus | Streptococcus suis\(^a\) | Escherichia coli K1 |
|------------------------|--------------------------|------------------------|-----------------------|--------------------------|-------------------|
| **Gram-positive cocci, encapsulated, serotype diversity, extracellular** | Gram-negative cocci, encapsulated, serogroup diversity, clonal complexes, extracellular | Gram-positive cocci, encapsulated, serotype diverse, Type III most common, extracellular | Gram-positive cocci, encapsulated, serotype diversity, extracellular | Gram-negative rod shaped, K1 capsular polysaccharide |
| **Affected age group** | Children <5 years
Adults >50 years | Children <5 years | <3 months
Adults | <3 months |
| **Site(s) of entry and colonization** | Nasopharynx, Lung | Nasopharynx | Hematogenous spread from mother to infant, nasopharynx, intestinal tract | Hematogenous spread from mother to infant, nasopharynx, intestinal tract |
| **Factors involved in bacterial adhesion and invasion** | Cell wall-anchored proteins, cytolysin, capsule | Capsule, type IV pili, outer membrane proteins (Opa, Opc, FBA, ACP, MspA) | Cell wall-anchored proteins, hemolysin, capsule, LTA, pili | OmpA, K1 capsule, CNF1, Fimbriae, IbeA |
| **Mechanisms of survival and dissemination in the blood** | Capsule-dependent protection, complement inhibitors | Capsule-dependent protection, complement inhibitors | Capsule-dependent protection, complement inhibitors, intracellular survival | Capsule-dependent protection, complement inhibitors, monocytes as “Trojan Horse” |
| **Mode(s) of entry into the CNS** | Invasion across the BBB and B-CSFB | Invasion across the B-CSFB | Invasion across the BBB and B-CSFB? | Invasion across the BBB and B-CSFB |
| **Causes of tissue damage in the CNS (cerebral ischemia, edema, hydrocephalus, increased intracranial pressure)** | Cytotoxin, cell wall-TLR2 induced inflammation, neuronal apoptosis, increased BBB permeability | Release of inflammatory mediators, increased BBB permeability, neuronal apoptosis, LPS | Hemolysin induced inflammation, tight junction disruption, increased BBB permeability | Release of inflammatory mediators, increased BBB permeability, neuronal apoptosis? |
| **Pathology and clinical symptoms** | Meningitis, sepsis, pneumonia | Meningitis, sepsis | Meningitis, sepsis, pneumonia | Meningitis, endocarditis, peritonitis, pneumonia, arthritis, sepsis, STSLS |
| **Possible sequelae** | Deafness, learning deficits, paralysis | Deafness, neuro-developmental Learning deficits, deafness, cortical blindness, seizures | Deafness | Deafness |

*BBB* blood–brain barrier, *B–CSFB* blood–cerebrospinal fluid barrier, *STSLS* streptococcal septic shock-like syndrome, *LTA* lipoteichoic acid

\(^a\) *S. suis* can cause meningitis in pigs and humans. This table only shows features of human infections.
In the case of the pneumococcus, PCho is added to cell wall teichoic acid and lipoteichoic acid in a phase variable manner [22]. Binding of PCho to the PAFr leads to clathrin-mediated uptake of bacteria into a vacuole, thereby facilitating intracellular bacterial translocation from the bloodstream into the brain [103]. Experiments using PCho antagonists or PAFr-deficient mice revealed that bacteria fail to invade the bloodstream or CNS when this receptor is not available [36, 107]. The interaction of PCho with PAFr is counteracted by the host innate immunity components C-reactive protein (CRP) and surfactant, both of which target PCho [43]. The pneumococcus has also been described to use the vitronectin-αvβ3 integrin complex for invasion of epithelial and endothelial cells [9].

In addition to receptor-mediated uptake into host cells, the pneumococcus gains access into the CNS paracellularly by disruption of BBB integrity. This process is mediated by the cholesterol-dependent cytolsin pneumolysin [162] and the α-glycerophosphate oxidase GlpO [71] that creates H$_2$O$_2$ thereby causing apoptosis of brain microvascular endothelial cells. Hyaluronidase might also contribute to meningitis by degradation of components of the extracellular matrix [59]. Further, a secreted version of NanA appears to modulate tight junction protein expression by activation of TGF-β resulting in an increase of BBB permeability (unpublished results). But sustained bacteremia is not always a prerequisite for the pneumococcus to enter the CNS. In adults, meningitis can be caused by direct invasion from neighboring infected tissues. A recent study revealed that pneumococcal carriage in the nasopharynx can lead to pneumococcal invasion of the brain via retrograde axonal transport along olfactory neurons [146].

**Immune activation and inflammatory response in the brain**

Once the pneumococcus gains access to the CNS, it takes advantage of the limited host defense mechanisms in this compartment and rapidly multiplies within the cerebrospinal fluid (CSF). During multiplication, bacteria release components that are highly immunogenic and are recognized by pattern recognition receptors (PRRs) on the surface of antigen-presenting cells that are present in low numbers in the CSF. Immune recognition of these bacterial components results in a strong inflammatory response leading to BBB impairment due to recruitment of leukocytes (Fig. 1a), vascular deregulation, vasculitis and occlusion of vessels (Fig. 1c) which cause increased intracranial pressure. Interestingly, inflammation within the CNS is detectable at high titer bacteremia even prior to when bacteria cross the BBB [48].

The entire symptom complex of meningitis can be triggered in the absence of live bacteria, when only components of the bacterial cell wall are intracisternally inoculated into animals [141]. This observation is especially important in the clinical setting since bacterial lysis caused
by antibiotic treatment leads to explosive cell wall release, resulting in an increased host response and disease severity [86]. The most important PRRs responsible for the detection of the pneumococcus in the CNS are members of the Toll-like receptor (TLR) family (TLR2, TLR4 and TLR9) and NOD2 that belongs to the family of NOD-like receptors (NLRs) [82]. TLR2 recognizes pneumococcal cell wall, lipoproteins as well as lipoteichoic acid, whereas TLR4 detects pneumolysin and TLR9 senses bacterial DNA that is released during autolysis [58]. In addition, muramyl peptides from pneumococcal peptidoglycan are recognized by intracellular NOD2 [69] and PCho-bearing teichoic acids bind to the PAFr [21]. Inflammmasome-mediated recognition of the pneumococcus also contributes to the host innate immune response. The inflammasome component NALP3 has been shown to be a critical player in this process [45].

Engagement of the inflammatory response activates various signaling cascades resulting in the production of pro-inflammatory mediators that orchestrate an efficient immune response. Patients with pneumococcal meningitis show high levels of pro-inflammatory cytokines such as TNF-α, IL-1β, IFN-γ, IL-2, IL-6 and IL-12, anti-inflammatory cytokines (IL-10 and TGF-β) and chemokines such as CXCL8 (IL-8) CCL3 (MIP-1a) and CCL2 (MCP-1) in their CSF [19]. The secreted chemokines act together with other chemoattractants (e.g., PAF, reactive oxygen and nitrogen species) and the complement system to attract highly activated PMNs to the brain. These cells cross the BBB through the tight junctions of the endothelial cells that form this barrier in a multistep process involving integrins and selectins, leading to CSF pleocytosis [82]. Matrix metalloproteases (MMPs) produced by neutrophils, neurons, glia cells and endothelial cells upon infection have been shown to play an important role in this process by lysing the subendothelial basement membrane thereby promoting BBB breakdown and leukocyte invasion [67]. However, the invading leukocytes present in the CSF do not efficiently phagocytose the pneumococcus. This might partly be due to the lack of sufficient concentrations of complement components and immunoglobulin to opsonize the pathogen.

Activation of the immune response and the rapid influx of leukocytes into the brain also come at a cost for the host. Activated immune cells within the brain, such as microglia, astrocytes and infiltrating leukocytes as well as microvascular endothelial cells, amplify the cascade of pro-inflammatory cytokines and cytotoxic agents that cause tissue damage in cortical and subcortical structures [82]. Inhibition of many steps in the inflammatory cascade, such as neutrophil recruitment, improves the clinical outcome of meningitis by decreasing neuronal loss [5]. Therefore, antibiotic treatment of community-acquired meningitis is most often accompanied by administration of dexamethasone, to protect the brain from the abrupt increase of inflammation during early bacterial death.

Meningococcal meningitis

Neisseria meningitidis (meningococci) is a frequently found asymptomatic colonizer of the upper respiratory tract, which under certain circumstances may penetrate the mucosal membrane, reach the bloodstream and cause severe septicemia and/or meningitis. The interaction of N. meningitidis with human endothelial cells lining the blood vessels of the blood–CSF barrier (B-CSFB) is a prerequisite for the development of meningitis. Over the past decade, important advances have been made in understanding the molecular mechanisms of the interaction of N. meningitidis with endothelial cells of the B-CSFB. The following chapter will highlight the current knowledge about the specific adhesion-receptor interactions that allow N. meningitidis to tightly bind to the targeted host cell with a focus on the induced signaling pathways.

Bacterial invasion and dissemination

Bacterial binding to brain endothelial cells is a prerequisite for successful penetration into the CSF. Large colonies of N. meningitidis have been found on the capillaries of the subarachnoid space, in the parenchyma and in the choroid plexus in histological sections of brain tissues of post-mortem samples [100]. To establish binding to host cells, N. meningitidis possess a variety of determinants that contribute to these interactions including type IV pili, outer membrane proteins (Opa and Opc), and a number of newly described so-called minor adhesion or adhesion-like proteins, such as the adhesin complex protein (ACP) or the autotransporter meningococcal serine protease A (MspA) (for a review see [148]).

Type IV pili (Tfp) are polymeric filaments that are found in a variety of Gram-negative bacteria. They mediate the initial contact of N. meningitidis to eukaryotic cell surfaces, and are involved in bacterial movement, also known as ‘twitching motility’, and transformation competence. Tfp in Neisseria spp. are composed of one main component, the major pilin, PilE, that assembles into a helical fiber. The helical assembly of pilin into fibers relies on proteins located in or in the vicinity of the cytoplasmatic membrane.

Considerable efforts have been undertaken to determine the binding receptor of Tfp on eukaryotic cells. CD46 or membrane co-factor protein has been described as a proposed host cell receptor for Tfp [51], but the role of CD46 as a host cell receptor has been controversial. In addition, the platelet activating factor (PAFr) was described as a pilus receptor targeted on airway epithelial cells [49].
Recent published data now shed new light on a possible pilus receptor targeted on brain endothelial cells. Bernard et al. [11] showed that \textit{N. meningitidis} utilizes CD147, a member of the immunoglobulin superfamily, for Tfp-dependent adhesion to endothelial cells and demonstrated the central role of CD147 for vascular colonization of pathogenic meningococci. Tfp-mediated adhesion to CD147 was shown to involve both PilE and the minor pilin PilV. Interfering with Tfp/CD147 interaction blocked binding of meningococci to human endothelial cells in vitro and importantly also prevented colonization of vessels in human brain tissue explants ex vivo [11]. Furthermore, PilE- and PilV-dependent colonization of \textit{N. meningitidis} to endothelial vessels was verified in vivo using a model of severe combined immunodeficiency mice grafted with human skin [11]. Interestingly, both pilins have also been reported to activate the G protein-coupled β2-adrenergic receptor (β2-AR) that serves primarily as a signaling receptor [17]. In response to bacterial adhesion and the formation of meningococcal microcolonies, β2-AR is recruited to the apical surface of the endothelial cell underneath the microcolonies [17]. The interaction of PilE and PilV with the extracellular N-terminal domain of β2-AR most likely modifies the conformation of the receptor resulting in the activation of β-arrestin-mediated signaling pathways [17]. However, \textit{N. meningitidis}-induced activation of β2-AR does not elicit G protein-mediated signal transduction. The receptor activation by meningococci is biased toward the β-arrestin pathway. Trapped β-arrestin recruits ezrin and the non-receptor tyrosine kinase (RTK) c-Src, which phosphorylate cortactin (Fig. 2). Secondly, β-arrestin leads to the accumulation of β-arrestin-interacting proteins, such as VE-cadherin and p120-catenin, into so-called ‘cortical plaques’ underneath bacterial microcolonies. This accumulation was shown to result in depletion of intercellular junctions, a mechanism described in more detail below.

The outer membrane proteins comprise the colony opacity-associated (Opa) proteins and Opc. Though outer membrane proteins are partially masked by the polysaccharide capsule, they also efficiently support adhesion and invasion to eukaryotic cells especially on cells of high receptor density as would be induced in inflammatory conditions and/or lateral receptor aggregation [12]. Most Opa proteins have been demonstrated to bind to members of the human carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family on epithelial cells (for reviews see [111]). In addition, some Opa proteins can bind to heparan sulfate proteoglycans (HSPG) or to integrins via the extracellular matrix proteins vitronectin and fibronectin or saccharides [147]. Although binding of the Opa proteins to CEACAM receptors has been described in detailed for epithelial cells, there is only limited information about the role of CEACAMs on brain endothelial cells and the contribution of the Opa/CEACAM receptor interaction during meningococcal adhesion and/or invasion into brain endothelial vessel cells.
The outer membrane protein Opc is particularly implicated in host cell invasion of endothelial cells, including brain endothelial cells [148]. Opc is a beta barrel protein with five surface loops encoded by a single gene (opcA) and is antigenically stable. The level of Opc protein expression is phase variable, due to the transcriptional regulation of a homopolymeric polycytidine (Poly-C) stretch, within the promoter region [112]. The number of nucleotide repeats determines the promotor strength and binding efficacy of the RNA polymerase. Opc is expressed by several virulent N. meningitidis lineages, but is absent from certain epidemic clones (ET-37/ST-11 clonal complex) and a few random endemic isolates [112]. Interestingly, two epidemiological studies reported outbreaks where meningococcal strains of the ST-11 complex tend to cause severe sepsis [112].

The Opc protein can bind directly to components of the extracellular matrix (ECM) and serum proteins, such as vitronectin or fibronectin [110, 143]. In addition, Opc may indirectly bind to fibronectin via heparin, since both fibronectin and vitronectin are heparin-binding proteins. By binding to fibronectin or vitronectin bacterial adhesins can also target proteoglycans. The tight association of Opc to vitronectin and/or fibronectin in turn mediates binding of meningococci to their cognate receptor, endothelial αVβ3 integrin (vitronectin receptor) [110] and/or α5β1-integrin (fibronectin receptor) [143] on brain vessel cells.

Besides the activation of the non-RTK c-Src in a Tfp-dependent manner, meningococcal binding to integrins via Opc also leads to activation of c-Src. Detailed analysis revealed that pharmacological inhibition of c-Src activity as well as genetic interference with c-Src expression interfered with bacterial uptake [125]. The role of this kinase in bacterial uptake was further verified in Src-deficient fibroblasts that are impaired in their ability to internalize N. meningitidis. Similar to the role of c-Src, pharmacological inhibition and genetic ablation of the focal adhesion kinase (FAK) also blocked bacterial uptake [124]. As a downstream target cortactin is phosphorylated downstream of integrin-Src activation, demonstrating that a cooperative interplay between FAK, Src and cortactin occurs during meningococcal uptake by brain endothelial cells (Fig. 2) [124].

Bacteriological studies reported outbreaks where meningococcal adhesins/invasins from the cell periphery and is cleaved to a smaller sized involved in the release of cytokines and chemokines: this is evidenced for example for the N. meningitidis infection of the cell line HBMEC, which requires c-Jun kinases 1 and 2 (JNK1 and JNK2) activation for bacterial uptake, but not for cytokine release. Cytokine release instead, such as IL-6 and IL-8 from infected HBMEC involves the p38 mitogen-activated protein kinase (MAPK) pathway [128].

**Bacterial translocation into the CNS**

The tight interactions of the bacterial adhesins/invasins with their respective receptors on brain endothelial cells and subsequent induced uptake favor the strategy for a transcellular pathway for meningococcal transversal across the tight B-CSFB. A paracellular pathway would require opening of the tight junctions or even breakdown of the barrier as a consequence of induced apoptosis or cytotoxicity. The latter is unlikely, since subarachnoid hemorrhage is a rare complication of bacterial meningitis. Recent publications have highlighted mechanisms that facilitate a paracellular route for N. meningitidis translocation into the CNS [18, 114]. When adhering to endothelial cells, N. meningitidis induces local elongation of the cell resembling epithelial microvilli structures [33]. These microvilli-like structures surround the bacteria and initiate their internalization within vacuoles [33]. They increase the cell membrane surface to facilitate bacterial adhesion and contribute to resistance against shear stresses in the bloodstream [72].

Interestingly, formation of these cellular protrusions was also observed ex vivo in histological section of a choroid plexus capillary from a postmortem sample [85]. These protrusions are enriched in ezrin and moesin, two members of the ezrin–radixin–moesin (ERM) protein family, and several transmembrane proteins, including ICAM-1, ICAM-2 and CD44 [33]. Recruited integral membrane proteins, adapter proteins and the actin cytoskeleton form specific molecular complexes also referred to as ‘cortical plaques’. Interestingly, as a result of the formation of ‘cortical plaques’ replacement of proteins usually localized at the intercellular junctions occurs. In particular, the polarity complex PAR3/PAR6/αPKC proteins are recruited at the meningococcal adhesion site [18] with depletion at the cell–cell interface and opening of the intercellular junctions of the brain–endothelial interface. The formation of the misplaced adherence junctions may open up a paracellular route for N. meningitidis transversal into the CNS [18].

Further altering of cellular junctional proteins in vitro has been shown for the tight junction protein occludin using the HBMEC cell line as an in vitro model [114]. Prolonged time of infection resulted in proteolytic cleavage of occludin by the matrix-metalloproteinase MMP-8 [114]. As a consequence of proteolytic cleavage occludin disappears from the cell periphery and is cleaved to a smaller sized
50-kDa protein in infected cells resulting in endothelial cell detachment and increased paracellular permeability [114].

Bacterial binding and subsequent uptake by the host cells not only implicates binding to specific ligand receptor, but requires a re-organization of receptor molecules and of signaling molecules in the cell membrane. Recent studies indicate that specialized domains of the cell membrane, termed rafts, are central for the spatial organization of receptors and signaling molecules. Bacteria can hijack and take advantage of these signaling platforms activated within specialized membrane domains.

Studies in the last years revealed that lipids in the cell membrane are not randomly distributed but seem to be organized. Sphingomyelin is the most prevalent sphingolipid and predominantly localizes in the anti-cytoskeletal leaflet of cell membranes and intracellular vesicles. It is composed of a highly hydrophobic ceramide moiety and a hydrophilic phosphorylcholine headgroup. Hydrolysis of sphingomyelin results in the release of ceramide which alters the biophysical properties of membranes. Ceramide molecules spontaneously interact with each other to form ceramide-enriched domains and, due to their biophysical properties, ceramide-enriched membrane domains then fuse into extended platforms which span a few hundred nanometers to several micrometers. In addition to altering membrane fluidity and rigidity, ceramide-enriched platforms serve to sort and eventually concentrate membrane receptors and membrane proximal signaling components thereby amplifying cellular responses and signal transduction. Ceramide-enriched platforms have been implicated in the internalization of different bacteria [44]. Recent published data now revealed that N. meningitidis is also capable to activate the acid sphingomyelinase (ASM) in brain microvessels thus leading to generation of ceramide and the formation of ceramide-enriched platforms [123]. Mechanistically, ASM activation relies on binding of N. meningitidis to its attachment receptor, HSPG, followed by activation of the phosphatidyicholine-specific phospholipase C. In addition, N. meningitidis infection promoted receptor (ErbB2) recruitment in ceramide-enriched platforms. Interestingly, meningococcal isolates of the ST-11 clonal complex, which rarely cause meningitis (see above), barely induced ASM and ceramide release correlating with significant lower bacterial uptake by brain endothelial cells [123]. These data indicate a differential activation of the ASM/ceramide system by the species N. meningitidis determining its invasiveness into brain endothelial cells.

Immune activation and inflammatory response in the brain

Cytokine activation is an important event in the pathogenesis of meningococcal disease [149]. The acute inflammatory response is compartmentalized within the subarachnoid space and is characterized by the release of tumor necrosis factor α (TNF-α), IL-1β, IL-6, IL-8, MCP-1, MIP-α and G-CSF [149]. Interestingly, based on experiments with meningioma cells, N. meningitidis induce higher levels of the cytokines than the same number of S. pneumoniae, H. influenzae or E. coli K1 [46]. LPS is the major inflammatory modulin produced by N. meningitidis, however, several studies have shown that non-LPS components also contribute to cytokine secretion. The release of cytokines results in alteration of the vasculature of the meninges and in upregulation of different adhesion molecules on the endothelial cells, including selectins, intercellular adhesion molecules (ICAMs) and the vascular endothelial adhesion molecules (VECAMs). Circulating leukocytes, primarily neutrophils, are attracted by IL-8 and can pass between the activated endothelial cells entering the subarachnoid space. In parallel, proteins (mainly albumin), immunoglobulins and complement factors leak into the CSF. TNF-α and IL-1β are produced at the very early stage and can be found in a bioactive form in half of the patients on admission. The release of IL-6, IL-8, MCP-1 and MIP-α continues for a longer time or are upregulated to higher levels and can be detected in the majority of the patients during hospital admission.

Group B Streptococcus meningitis

Group B Streptococcus (GBS) is a Gram-positive encapsulated bacterium possessing an array of virulence factors that enable it to produce serious disease in susceptible hosts, in particular the human newborn [73]. Notably, GBS is the leading cause of meningitis in the neonatal period [73]. Although advances in intensive care management and antibiotic therapy have changed GBS meningitis from a uniformly fatal disease to a frequently curable one, the overall outcome remains unfavorable. Morbidity is high with 25–50 % of surviving infants suffering permanent neurological sequelae, including cerebral palsy, mental retardation, blindness, deafness, or seizures [32]. The pathogenesis of neonatal GBS infection begins with the asymptomatic colonization of the female genital tract. Approximately 20–30 % of healthy women are colonized with GBS on their vaginal or rectal mucosa, and 50–70 % of infants born to these women will themselves become colonized with the bacterium [3]. Of the 10 different GBS capsular serotypes described, five (Ia, Ib, II, III, and V) are typically more associated with disease and account for the majority of cases worldwide [31]. GBS has more recently also been classified by sequence type (ST) based on an allelic profile of seven different loci, with the majority of GBS human isolates being ST-1, ST-17, ST-19, or ST-23 [50]. Interestingly there is a disproportionate burden of
serotype III, ST-17 strains associated with neonatal invasive disease and meningitis [136]. The type III, ST-17 GBS clone has been referred to as the hypervirulent strain and accounts for the majority of GBS meningitis cases [136]. In this section, we review the mechanisms by which GBS is able to gain access to, and penetrate the BBB as well as highlight the response of the BBB to GBS with particular emphasis on newly described mechanisms of GBS BBB penetration.

Neonatal GBS infections are traditionally classified as two forms: early-onset disease (EoD) and late-onset disease (LoD). Early-onset infections typically occur in the first week of life, presenting acutely with pneumonia and respiratory failure complicated by bloodstream infection, sepsicaemia and sometimes meningitis. In contrast, GBS LoD occurs in infants up to 7 months of age, with more indolent symptom progression related to bacteremia and a high incidence (~50 %) of meningitis [3]. The pathophysiology of GBS meningitis varies according to age of onset. In EoD, autopsy studies demonstrate little or no evidence of leptomeningeal inflammation, despite the presence of abundant bacteria, vascular thrombosis and parenchymal hemorrhage [102]. By contrast, infants with LoD usually have diffuse purulent arachnoiditis with prominent involvement of the base of the brain [10]. These histopathological differences reflect underdevelopment of the host immunological response in the immediate neonatal period, with a higher proportion of deaths resulting from overwhelming septicemia. Clinical and neuropathologic studies have documented the clear association between bacterial meningitis and brain edema formation, increased intracranial pressure, seizure activity, arterial and venous cerebral vascular insults, and other neurologic sequelae [113]. A recent study found that GBS meningitis can be complicated by severe cerebrovascular disease, including arterial ischemic stroke and cerebral sinovenous thrombosis, and that these complications may be underestimated [140].

To produce meningitis, blood-borne GBS must typically penetrate the BBB and/or the B-CSFB. Ultimate disruption of BBB integrity may be due to the combined effect of bacterial entry and penetration of brain microvascular endothelial cells (BMEC), direct cellular injury by bacterial cytotoxins, and/or activation of host inflammatory pathways that compromise barrier function. It is apparent that the host immune response is incapable of controlling infection within the CNS and that this host inflammatory response may be responsible for many adverse events during bacterial meningitis. A very complex and integrated series of events involving host cytokines, chemokines, proteolytic enzymes, and oxidants appears to be responsible for meningitis-induced brain dysfunction. The development of GBS meningitis progresses through phases including (1) bloodstream survival and the development of bacteremia, (2) direct GBS invasion and disruption of the BBB, and (3) GBS multiplication in the CSF-containing subarachnoid and ventricular spaces, which induces inflammation with associated pathophysiologic alterations leading to the development of neural damage.

**Bacterial invasion and dissemination**

An association between sustained high-level bacteremia and development of GBS meningitis has been suggested in humans and in experimental models of hematogeneous meningitis [73]. This observation implies that GBS bloodstream survival is an important virulence trait to avoid immune clearance by host immune cells, prior to CNS penetration. Neonates are particularly prone to invasive disease because of their quantitative or qualitative deficiencies in phagocytic cell function, specific antibody, or the classic and alternative complement pathways. In addition to these newborn host susceptibilities, GBS possess a number of virulence determinants that promote bloodstream survival by thwarting key components of effective opsonophagocytic killing by host leukocytes [73]. The sialylated GBS capsular polysaccharide (CPS) represents one of the most critical factors for limiting the effectiveness of host complement and phagocytic defense. While serotype III GBS strains have accounted for a majority of LoD and meningitis [3, 136], all serotypes contain a terminal-linked sialic acid bound to galactose in an α2 → 3 linkage [73]. Bacterial surface sialylation may have evolved to mimic host ‘self’ antigens, allowing GBS to avoid immune detection, manipulate phagocyte function and dampen the immune response to GBS infection. The sialic acid moiety provides anti-phagocytic protection by impairing deposition of opsonically active complement C3 on the bacterial surface, but also activates anti-inflammatory receptors on host leukocytes promoting GBS persistence in the blood stream [73]. Isogenic GBS mutants lacking CPS or capsular sialylation are more susceptible to neutrophil killing and are less virulent in rodent and zebrafish infection models [93, 109].

Once GBS is engulfed by phagocytic cells, the bacterium may be able to resist toxic reactive oxygen species (ROS) produced in the phagolysosome to survive intracellularly. GBS produces an orange carotenoid pigment, a property unique to GBS among hemolytic streptococci, associated with the cyl operon encoding the β-hemolysin/ cytolsin cytotoxin [73]. The free-radical scavenging properties of this associated carotenoid neutralize hydrogen peroxide, superoxide, hypochlorite and singlet oxygen, and thereby provide a shield against several elements of phagocyte ROS killing [68]. GBS transcriptional regulators CovR [20] and CiaR [101] have also been linked to survive inside phagocytic cells, likely acting to coordinate expression of acid and stress survival genes.
Bacterial translocation into the CNS

Following bloodstream survival, GBS interacts directly with BBB endothelium, which can result in bacterial invasion of the BBB with subsequent infection of the CNS. This process can result from increased permeability of the BBB and/or the direct invasion of BMEC by the pathogen (Fig. 3). With the availability of in vitro tissue culture models of human BMEC (HBMEC) and animal models of GBS infection, significant progress has been made identifying and characterizing the molecular determinants that promote GBS–BBB interaction. GBS enter or “invade” brain endothelium apically and exit the cell on the basolateral side, thereby crossing the BBB transcellularly [90]. Electron microscopic (EM) studies have demonstrated the presence of the meningeal pathogen in membrane-bound vacuoles within HBMEC [23, 90], suggesting the involvement of endocytic pathways as well as avoidance of lysosomal fusion for BBB traversal. This process may be accomplished, at least in part, by tyrosine phosphorylation of focal adhesion kinase (FAK), which occurs upon GBS infection. Phosphorylation of FAK induces its association with PI3K and paxillin, an actin filament adaptor protein, and is required for efficient GBS HBMEC invasion.

To elucidate the GBS determinants involved in the pathogenesis of meningitis, many groups have focused on the characterization of serotype III, ST-17 GBS isolates responsible for CNS disease. Screening of a GBS ST-17 mutant library revealed a unique requirement for the novel “invasion associated gene”, iagA, in BBB penetration by GBS [29]. Decreased invasion of HBMEC by the GBS ΔiagA mutant in vitro was correlated with a reduced risk for development of meningitis and markedly diminished lethality in vivo. The iagA gene encodes an enzyme for biosynthesis of diglucosyldiacylglycerol, a membrane glycolipid that functions as an anchor for lipoteichoic acid (LTA), indicating that proper LTA anchoring is important to facilitate GBS BBB penetration [29]. Interestingly, clinical GBS isolates from infants with EoD or LoD possess higher quantities of cell-associated LTA than strains isolated from...
mucosal surfaces of asymptomatically colonized infants [89]. The availability of GBS genome sequences has enabled the identification of genes restricted to the ST-17 lineage. One gene, now called hypervirulent GBS adhesion (HvgA), was shown to be required for GBS hypervirulence [136]. GBS strains that express HvgA are more efficient in crossing the intestinal and blood-brain barriers in neonates, including choroid plexus epithelial cells and brain microvascular endothelium [136].

Proteins targeted for cell surface expression in GBS are predicted to share a C-terminal sequence (L/IPXTG) for sortase recognition and anchoring to the Gram-positive cell wall. In a paradigm-shifting study, it was discovered that GBS express cell wall-anchored pili [65]. Among the sequenced GBS genomes, two genetic loci encoding pili have been identified, Pilus Island (PI)-1 and PI-2, the second existing in one of two variants (PI-2a and PI-2b), and not all genomes contain both loci [73]. GBS PI-2a includes the genes encoding PilB, an LP(x)TG-motif-containing protein that polymerizes to form a pilus backbone, and accessory pilus proteins PilA and PilC that are incorporated in the pilus [73]. Both PilA and PilB promote adherence to and invasion of brain endothelium, respectively [74]. It has been demonstrated that PilA binds the extracellular matrix (ECM) component, collagen, and that collagen binding enhanced GBS attachment as well as uptake into HBMEC in a dose-dependent manner [4]. Further, the PilA-collagen complex engages α2-β1 integrins on brain endothelium to promote bacterial attachment and pro-inflammatory chemokine release [4]. As a result, increased neutrophil infiltration was correlated with increased BBB permeability and higher levels of bacterial CNS penetration in vivo [4].

In addition to PilA, other GBS factors interact with various ECM proteins and constituents to promote bacteria-BBB interactions. The GBS surface anchored alpha C protein (APC) was shown to interact directly with glucosaminoglycans (GAGs) on brain endothelium, and promote the establishment of GBS meningitis [15]. More recently, a GBS fibronectin-binding protein, Streptococcal fibronectin-binding factor A (SfbA), was shown to contribute to GBS invasion of HBMEC in vitro and to the development of meningitis in vivo [84]. Interestingly, studies have suggested that adherence to fibrinogen may be a general property of GBS to promote bloodstream survival and host cell interactions [120]. An important determinant recently implicated in fibrinogen binding and BBB interaction is the GBS serine-rich repeat (Srr) glycoprotein [120]. GBS strains carry 1 of 2 srr gene alleles, designated srr1 and srr2, which are similar in architecture but show only limited homology (<20 % identity). Expression of the Srr-2 protein seems to be restricted to serotype III and ST-17 strains. Recent structural studies demonstrated that both Srr1 and Srr2 interact with tandem repeats of the fibrinogen α chain via a “dock, lock, and latch” mechanism [119]. Moreover, increased affinity between Srr2 and fibrinogen was observed, suggesting that a greater affinity for fibrinogen may contribute to the increased virulence associated with Srr2-expressing strains [119].

**Immune activation and inflammatory response in the brain**

The host inflammatory response to GBS contributes significantly to the pathogenesis of meningitis and CNS injury. The first comprehensive microarray analysis of the BBB endothelium transcriptional response to a bacterial pathogen was examined during GBS infection [30]. Highly induced genes were those involved in the inflammatory response, including Interleukin (IL)-8, CXCL1, and CXCL2, ICAM-1, and GM-CSF, which function to orchestrate neutrophil recruitment, activation and enhanced survival [30]. Several studies have shown an association between leukocyte trafficking and BBB permeability and increased GBS penetration of the CNS, suggesting that PMN-mediated damage of the BBB has a significant role in the pathogenesis of GBS meningitis [4, 30]. It is clear that the GBS β-haemolysin/cytolysin (β-h/c) toxin contributes to immune activation and much of the observed disease pathology. Hemolysin expression has been shown to directly damage brain cells including brain endothelial cells [90], leptomeninges (meningioma cells) and astrocytes [2], and primary neurons [106]. Further, toxin expression was identified as a principal provocative factor for BBB activation, contributing to the development of meningitis [30]. Recently GBS β-h/c was also shown to activate autophagy in BBB endothelium [23]. Although results demonstrated that antibacterial autophagy provided a BBB cellular defense against invading and toxin producing bacteria, GBS was not completely eliminated, suggesting that GBS may actively thwart the autophagic pathway [23].

Microarray analysis of brain endothelium has also indicated that HBMEC respond to GBS infection by upregulating Snail1, a global transcriptional repressor of tight junction proteins [52]. Recent studies have demonstrated that during GBS infection transcript and protein levels of tight junction components ZO-1, Claudin-5 and Occludin were decreased in vitro in HBMEC and in vivo using murine and zebrafish models of GBS infection [52]. This was dependent on Snail1 induction, which was sufficient to facilitate tight junction disruption, promoting bacterial passage and disruption of the BBB [52]. Interestingly host integrins, ECM components and glycosaminoglycans involved in GBS-BBB interactions all preferentially localize to the basolateral surface of polarized endothelium. The subsequent loss of tight junctions may represent the critical
first step to disrupting cell polarity that enables bacterial pathogens like GBS to engage basolaterally expressed host receptors and promote BBB permeability and progression to meningitis.

**Streptococcus suis meningitis**

*Streptococcus suis* is one of the most important porcine bacterial pathogens responsible for high economic losses in the swine industry worldwide. It causes a wide variety of diseases, including meningitis, septicaemia and endocarditis. Among the 33 serotypes originally described based on CPS antigens, serotype 2 is not only prevalent in swine diseases but is also considered to be an emerging zoonotic agent causing meningitis and streptococcal toxic shock-like syndrome in humans [42]. *S. suis* gained more attention since recent recognition of its high prevalence in human meningitis cases in South East and East Asia, and reports of outbreaks which resulted in high mortality rates [151]. Patients suffering from *S. suis* meningitis have cerebrospinal fluid with high numbers of neutrophils. One of the most striking sequel of *S. suis* meningitis is the establishment of deafness and/or vestibular dysfunction. In fact, the incidence of deafness following infection caused by this pathogen is consistently higher than that reported for other meningitis-causing bacteria, such as *S. pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenza*. Following, host–pathogen interactions in the establishment of *S. suis* meningitis are summarized (depicted as a model in Fig. 4).

![Fig. 4 Pathogenesis of Streptococcus suis meningitis. 1 ApuA degrades glycogen and mediates adhesion to mucus. 2 S. suis harbors the cholesterol-dependent cytolysin SLY, which induces pore-formation in eukaryotic cells. 3 For a more effective adhesion and invasion, S. suis actively downregulates its polysaccharide capsule (CPS). 4 S. suis co-opts host proteins, such as serum and/or extracellular matrix (ECM) proteins and specifically interacts with epithelial cells by molecular bridges (e.g., with integrins). 5 S. suis evolves the proteases IGA1 and IdeSuis, which inactivate IgA and IgM, respectively, and thus prevents opsonization. 6 The Arginine Deiminase System (ADS) facilitates bacterial survival under acidic (intra-phagolysosomal) conditions in myeloid and non-myeloid cells. 7 CPS expression depends on nutrient availability and is high in blood but low in CSF. 8 Neutrophil Extracellular Trap (NET) formation is an ancient mechanism to combat bacterial infection. S. suis harbors to DNAses to circumvent NETosis. 9 S. suis uses monocytes to for dissemination. 10 S. suis-activated monocytes upregulate cellular adhesion molecules to interact with BMECs. 11 During infection, granulocytes overcome the B-CSFB by transmigration, thus serving as a vehicle for *S. suis* to disseminate into the CSF. 12 Upon *S. suis* infection, microglia upregulate innate immune pattern recognition receptors, such as TLR2, TLR3, CD14 and NOD2.](image-url)
Bacterial invasion and dissemination

As an opportunistic pathogen, *S. suis* colonizes the mucosal surfaces of the oropharyngeal and gastrointestinal tract of swine without inducing any clinical symptoms. However, since the mucosa constitutes a physical and immunological barrier to protect the host from invading pathogens, homeostasis between bacterium and host is a prerequisite for stable colonization. Additionally, inter- and intrabacterial competition for nutrients might also determine the success of an opportunist to permanently populate its preferred host. On the other hand, breakage of the epithelial barrier is often required for bacterial dissemination into deeper tissue sites. How *S. suis* interferes with the immune system of the mucosa and facilitates epithelial transmigration is only poorly characterized. Ferrando et al. [35] identified ApuA, an amylolpullulanase with α(1,4)- and α(1,6)-glycolytic activity that allows *S. suis* to degrade glycogen and food-derived starch under in vivo conditions. Furthermore, ApuA mediates adhesion to mucus and, thus, displays an initial step in bacterial colonization. Immunoglobulins (Igs), such as IgA and IgM, are constituents of mucosal surfaces. By specifically coating the bacteria, Igs shape the microbiome and are involved in maintaining the bacteria-host homeostasis. *S. suis* has evolved two enzymes which specifically interact with mucosa-associated Igs. The IgA1 protease IGA is expressed in vivo and specifically cleaves IgA. Furthermore, its presence is strongly correlated with an invasive phenotype of *S. suis* suggesting an important role in pathogenesis [158]. Recently, the surface-associated IgM protease IdeSsuis was identified in a highly pathogenic strain. IdeSsuis specifically cleaves porcine IgM in vivo and, thereby, evades opsonization and complement-mediated killing when reaching the blood stream (see below) [115].

To reach systemic sites, *S. suis* has to breach the epithelial barrier. This may occur by different processes depending on expression of the CPS. Adhesion and invasion are significantly enhanced in unencapsulated isolates which is probably the result of a better accessibility of bacterial adhesins and invasins [8]. A variety of different bacterial cell-interacting proteins have been described (for review see [6]). Interestingly, in a recent study, Meng et al. [77] showed that capsule-dependent adhesion seems to be abrogated in co-infections of *S. suis* with highly pathogenic swine influenza virus. The underlying mechanisms, though not known in detail, might be based on different cellular receptor expression in complex primary multi-cellular precision-cut lung slices as compared to immortalized epithelial cell lines. In addition, interaction between the bacterium and the epithelial cell could also be of indirect nature. By co-opting host proteins of the extracellular matrix or serum proteins, *S. suis* is able to use them as a molecular bridge for adherence and invasion to/in host cells by receptor-mediated mechanisms (reviewed in [37]).

Epithelial transmigration might also be facilitated by cellular damage. *S. suis* possesses a thiol-activated cytolytic protein, suilysin (SLY), which can induce pore-formation in cholesterol-containing eukaryotic membranes. However, since bacterial mutants defective in SLY are still able to disseminate in the host [70], SLY activity seems to be important but not essential for systemic *S. suis* infections. Recently we discovered that SLY can promote adherence and host cell invasion of *S. suis* and that these effects also occurred at sublytic toxin concentrations [116]. However, the underlying mechanisms of SLY-mediated effects in adherence and invasion are yet unknown.

In the subepithelial environment, *S. suis* faces changing nutritional and immunological conditions. For example, whereas the capsule hinders bacterial adhesion (and invasion) to epithelial cells, it is essential for survival in blood due to its strong anti-phagocytic properties. Moreover, CPS mediates *S. suis* evasion of opsonization by immunoglobulins and activities of the complement system. Finally, a lower phagocytosis inevitably leads to reduced pro-inflammatory response and, thus, to a diminished tissue destruction and recruitment of immune cells. The fact that the capsule hinders transepithelial migration but enhances bacterial survival in the blood strongly indicates a tight regulation of CPS expression during pathogenesis. Indeed, Wu et al. [157] reported an increase in CPS expression when bacteria were grown in blood. In contrast, CPS gene transcription was low when *S. suis* was cultured in CSF, a compartment which is poor in nutrients. Accordingly, genes involved in carbohydrate and amino acid transport and metabolism were highly transcribed under such circumstances. Willenborg et al. [154] described a direct link between carbohydrate metabolism and CPS expression. A lack in the Carbon Control Protein A, the central regulator of Carbon Catabolite Repression in Gram-positive bacteria, led to a low capsule expression and attenuated survival in the presence of primary phagocytes [154]. Accordingly, other studies also revealed a link between nutrient starvation and enhanced virulence properties of *S. suis*. Thus, further work on metabolic adaptation will surely contribute to a better understanding of the pathogenesis of *S. suis* infections.

Similar to GBS, highly virulent and zoonotic serotype 2 *S. suis* strains possess neuraminidase activity to terminally link the CPS chains with sialic acid. However, in contrast to GBS, the sialic acid of *S. suis* is not α(2,3)-, but α(2,6)-linked to galactose moieties [145]. Whether this different sialylation pattern has an impact on immune recognition has to be proven in further studies. In addition to CPS expression and modification, other factors might be involved in survival in blood and bacterial dissemination. For example,
modification of the bacterial cell wall by N-deacetylation of the peptidoglycan or D-alanylation of the lipoteichoic acid (LTA) leads to resistance against neutrophil-derived lysozyme and antimicrobial peptides [38, 39]. The generation of Neutrophil Extracellular Traps (NETs), an “ancient” antimicrobial mechanism of eukaryotic cells, is combated by *S. suis* with the expression of at least two different DNA-degrading enzymes. Consequently, inactivation of the extracellular *S. suis*-secreted nuclease A (SnA) and the endonuclease A (EndAsuis) led to a reduced bacterial survival after co-cultivation with porcine granulocytes [25, 26]. Nevertheless, despite these anti-phagocytic factors, *S. suis* cannot prevent uptake by neutrophils. Eventually, some bacteria will be phagocytosed and inactivated in acidified phagolysosomes. However, *S. suis* also evolved strategies to overcome such inhospitable conditions. The pathogen possesses an Arginine Deiminase System (ADS), which increases intracellular survival of *S. suis* by neutralizing the intraphagolysosomal pH [40]. The ADS is characterized as a metabolic enzymatic system, which catalyzes the degradation from arginine to ornithine and thereby producing ATP, citrulline, CO₂ and NH₄⁺. Thus, the ADS represents a multifunctional system important for bacterial metabolism and biological fitness in the host.

**Bacterial translocation into the CNS**

*S. suis* bacteremia might result in the establishment of meningo-encephalitis in men and swine. However, to finally reach the cerebrospinal space or the brain parenchyma, respectively, *S. suis* is faced with two different cellular barriers, the BBB and the B-CSFB. The BBB is composed of a non-fenestrated monolayer of BMEC, which separates the brain from the intravascular space. BMECs are highly polarized with an apical and basolateral site expressing different surface proteins. This might be the reason why contradictory in vitro studies revealed an effective adhesion but only a very low invasion of *S. suis* in porcine and human BMEC [7, 16]. The different kind of host cell interaction is further underlined by the fact that, in contrast to epithelial cells, the CPS seems to play only a minor role in the primary adhesion process [16]. Thus, alternative bacterial and/or cellular factors might be necessary to overcome the BBB. Nevertheless, similar to the interaction with epithelial cells, LPXTG-anchored surface proteins, lipoproteins as well as “moonlighting” proteins seem to be involved in binding and invasion of *S. suis* to BMEC to a certain extent (reviewed in [37]). BMEC respond to a *S. suis* infection by an upregulation of a variety of different cytokines and chemokines, such as IL-1, IL-6, IL-8, and TNFα [144]. Furthermore, Al-Numani et al. [1] showed an upregulation of the cellular adhesion molecules ICAM-1, CD11a/CD18 and CD11c/CD18 on human THP-1 monocytes upon *S. suis* infection. These stimulated monocytes exhibit a significantly increased adherence to endothelial cells, thus supporting the (modified) “Trojan horse” theory as a mechanism to overcome the BBB. However, although binding and invasion of *S. suis* to porcine monocytes was shown in vitro, in vivo evidence is still lacking.

In contrast to the BBB, the B-CSFB is a two-layer barrier made up of a fenestrated endothelium followed by the choroid plexus epithelial cells (CPEC). Significant work was done on the interaction of *S. suis* with human and porcine CPEC. Though it turned out that bacterial adhesion and invasion is similar to epithelial cells from other tissues, unique differences were observed in the preferred route of bacterial transmigration. *S. suis* adheres and invades CPEC significantly better when applied from the basolateral site than from the apical site [138]. This is most likely due to subcellular-specific receptor expression. Nevertheless, this in vitro phenotype reflects the in vivo situation where *S. suis* enters the cerebrospinal fluid from the blood via the plexus choroideus. The interaction of *S. suis* with CPEC goes along with distinct cellular and immunological responses. For example, infections with *S. suis* lead to rearrangements of tight junction proteins and induction of stress fiber formation, thus leading to a loss of barrier integrity and release of pro-inflammatory cytokines [137]. Expression of TNFα as well as cell adhesion molecules, such as VCAM-1 and ICAM-1, promotes adhesion and subsequent transmigration of PMNs through CPEC [152]. Interestingly, transmigration of PMNs occurs via the transcellular route. Since the authors also detected *S. suis* inside PMNs, the “Trojan horse” theory should be carefully revisited.

**Immune activation and inflammatory response in the brain**

The pathogenesis of *S. suis* in the brain and its subsequent interactions with intracranial immune cells is only poorly understood. Dominguez-Punaro et al. [27] reported multifocal lesions from all areas of the brain as well as the meninges in mice upon *S. suis* infection. Lesions were accompanied by positive bacterial antigen reactions in immune-histochemical analysis and enhanced pro-inflammatory cytokine expression, which could later be reconstituted in vitro by infection of murine microglial cells with pathogenic serotype 2 *S. suis* [27, 28]. Interestingly, two independent studies reported an upregulation of innate immune pattern recognition receptors, such as TLR2, TLR3, CD14 and NOD2 in microglia upon *S. suis* infection [28, 160]. The mechanisms and functional relevance are unknown, but this may be a hint towards an intracellular fate of *S. suis*. It seems that *S. suis* does not actively invade astrocytes. However, a CPS- and SLY-dependent
upregulation of pro-inflammatory cytokines in these cells was shown, a response that appears to be mainly TLR2 driven. Nevertheless, more detailed work is highly demanded to get better insights into the mechanism of S. suis–glial cell interactions.

**Escherichia coli** K1-induced neonatal meningitis

*E. coli* K1 (*E. coli*) is the second leading cause of meningitis in neonates, but it is the leading pathogen in low-birth weight infants. Despite the drop in mortality rates from 50 % in 1970 to <20 % currently, the morbidity rates remain unchanged even with the use of effective antibiotics and supportive care [41]. The ever increasing numbers of antibiotic-resistant *E. coli* strains make the situation worrisome. An astounding 30–58 % of survivors suffer from serious neurological complications such as mental retardation, hearing loss and cortical blindness [41]. Although removal of bacteria from the circulation is the mainstay of antibiotic use, the release of large quantities of endotoxin from the dead bacteria triggers a massive inflammatory response resulting in septic shock. The use of corticosteroids to reduce this inflammatory response is ineffective in alleviating the neurological deficits associated with this disease. Therefore, a comprehensive understanding of the pathogenesis of *E. coli* meningitis is critical for the development of new therapeutic strategies.

Among *E. coli*, K1 CPS-decorated strains, a polymer of sialic acid residues, predominantly cause neonatal meningitis [54]. Besides K1 CPS, *E. coli* contains several surface structures such as pili, lipopolysaccharide, and outer membrane proteins that potentially interact with host tissues during the establishment of meningitis. Outer membrane protein A (OmpA) is the major protein of *E. coli*, and it is structurally conserved throughout the evolution [95]. However, recent studies have shown that pathogenic *E. coli* show minor differences in the extracellular loops of OmpA compared to non-pathogenic strains [127]. Several studies have demonstrated that OmpA plays a significant role in the pathogenesis of various diseases [62]. Other virulence factors of *E. coli* include IbeA, IbeB, yiiIP, TraJ, aslA and cytotoxic necrotizing factor 1 (CNF-1) [53]. Here, we review the interactions of OmpA with various cells for binding to and invasion of *E. coli* and how they contribute to the pathogenesis of meningitis.

To gain insights into the pathophysiology of bacterial diseases, a careful selection and usage of animal models is clearly required. Newborn rat and mouse models have routinely used to study the pathogenesis of *E. coli*. These models mimic the human disease as they both depend on age for infection and cause the disease by hematogenous spread. The pathology of the brain in rats or mice is similar to infected humans showing edema, neutrophil infiltration, neuronal apoptosis and meningeal damage [80]. Therefore, the studies presented here are from in vitro experiments or very relevant newborn rat and mouse models.

**Bacterial invasion and dissemination**

The colonization of mucosa by *E. coli* followed by invasion and crossing of the epithelial surfaces is critical for eventual spreading to intravascular space. Hek protein expressed by *E. coli* mediates adherence to and invasion of epithelial cells by binding to heparin sulfate glycosaminoglycans [34]. Succeeding invasion of mucosal surfaces allows *E. coli* to disseminate via hematogenous spread at which stage the bacterium must avoid initial serum bactericidal activity. Complement activation results in opsonization of bacteria for the formation of membrane attack complexes on the surface of pathogens, which mediates bacteriolysis. Opsonization with complement proteins also presents the bacteria to immune cells for phagocytosis. The K1 CPS of *E. coli* is shown to be necessary for the survival of the bacterium in the blood [54]. Subsequent studies using OmpA− *E. coli* additionally revealed that lack of OmpA renders the bacterium serum sensitive [97]. The bactericidal activity of serum against OmpA− *E. coli* appears to be mediated by classical complement pathway. Follow-up studies revealed that OmpA of *E. coli* binds to C4-binding protein (C4 bp), a classical complement pathway regulator to block the complement cascade reaction, and thereby avoids bacteriolysis and recognition by immune cells [97]. OmpA bound C4 bp acts as a co-factor for Factor I to cleave both C3b and C4b, which are important to present the bacterium to phagocytes [156].

The survival of *E. coli* in PMNs appears to be the first step in the pathogenic process as PMN depletion prevents the onset of meningitis in newborn mice [81]. The expression of OmpA is critical for survival inside PMNs after phagocytosis as OmpA− *E. coli* failed to survive. The phagocytosis of OmpA− *E. coli* by PMNs produces an enormous amount of reactive oxygen species (ROS) [122]. In contrast, OmpA+ *E. coli* suppressed the release of ROS even in the presence of external stimuli such as LPS, indicating that *E. coli* overrides PMN machinery to prevent antimicrobial activity. Lack of other virulence factors such as S-fimbriae, IbeA, type-1 fimbriae and CNF-1 had no effect on the suppression of ROS production. Rac1, rac2 and gp91Phox, the components of NADPH oxidase, an enzyme complex required for the production of ROS, were suppressed by *E. coli* K1 at the transcriptional levels in PMNs [81].

Analysis of various surface receptors such as TLRs, Fc-gamma receptors and complement receptors on PMNs after infection with *E. coli* revealed that the bacterium increases
the expression of gp96, an Hsp90 β-form but had no effect on other surface structures [81]. Again the OmpA of E. coli interacted with gp96 for entry and survival in PMNs, whereas, in the absence of gp96 expression, phagocytosed bacteria were killed efficiently. Moreover, entry of E. coli mediated by OmpA and gp96 interaction requires for reducing the levels of ROS. Substantiating the role of gp96 in E. coli-induced meningitis, suppression of gp96 using in vivo siRNA in three-day-old mice rendered them resistant to infection and prevented the brain damage. These gp96 knockdown mice could not develop bacteremia levels required to cross the BBB, suggesting that E. coli survival in PMNs is a critical step during the initial phases of infection.

Since PMNs are short-lived cells dying predominantly by apoptosis (Fig. 1b), E. coli must have alternative routes for survival and multiplication in neonates to reach high-grade bacteremia. Phagocytosis assays using RAW 264.7 and primary macrophages revealed that E. coli enters, survives and multiplies inside the cells, whereas OmpA− E. coli were killed by the cells immediately [134]. Of note, macrophage-depleted newborn mice became resistant to E. coli infection despite the presence of PMNs, suggesting that macrophages also provide a niche for bacterial multiplication. In macrophages, OmpA of E. coli binds to the alpha chain of Fc-gamma receptor I (CD64), which in turn produces biopterin. The biopterin subsequently acts as a co-factor for more inducible nitric oxide synthase (iNOS) activation and produce greater amounts of NO, which triggers the expression of CD64 to the cells surface. Thus, more E. coli bind to the receptor and enter the macrophages. The OmpA-CD64-mediated entry also avoids the fusion of lysosome with endosome, thereby finding a niche for survival and multiplication. To prevent the hostile conditions for bacterial survival, E. coli also suppresses mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinases (ERK1/2), and p38, thereby the activity of nuclear factor-κB (NF-κB). This arm of signaling prevents the production of pro-inflammatory cytokines in macrophages. Red lines indicate inhibition of specific signaling pathway

---

**Fig. 5** Mechanisms involved in Escherichia coli K1 manipulation of macrophages. The outer membrane protein A (OmpA) of E. coli K1 interacts with chitobiose moieties (GlcNAc1-4GlcNAc) in CD64 for inducing actin rearrangements to the sites of bacterial attachment for internalization of E. coli. During this process, the intracellular domain of CD64 triggers the upregulation of B cell lymphoma-extra large (Bcl-XL), an anti-apoptotic protein by an unknown mechanism to prevent apoptosis of the infected macrophages. In addition, toll-like receptor 2 (TLR2) ligands such as peptidoglycan (PGN) interaction with TLR2 also induces inducible nitric oxide (NO) production by activation of iNOS. Parallel to Bcl-XL upregulation, OmpA interaction with CD64 also enhances guanidine cyclohydrolase I (GCH1), which in turn produces biopterin. The biopterin subsequently acts as a co-factor for more inducible nitric oxide synthase (iNOS) activation and produce greater amounts of NO, which triggers the expression of CD64 to the cells surface. Thus, more E. coli bind to the receptor and enter the macrophages. The OmpA-CD64-mediated entry also avoids the fusion of lysosome with endosome, thereby finding a niche for survival and multiplication. To prevent the hostile conditions for bacterial survival, E. coli also suppresses mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinases (ERK1/2), and p38, thereby the activity of nuclear factor-κB (NF-κB). This arm of signaling prevents the production of pro-inflammatory cytokines in macrophages. Red lines indicate inhibition of specific signaling pathway
infection of monocytes not only allows the bacteria to survive but also prevents the production of various cytokines and chemokines from the cells [118]. The blocking effect of pro-inflammatory cytokines by E. coli is due to the degradation of IκB followed by inhibition of NF-κB activity. Furthermore, E. coli controls ERK1/2 and p38 MAP kinases by modulating their phosphorylation status, and thus regulating IκB degradation. In that context, infection of three-day-old mice triggered the production of IL-10 at early stages of infection, indicating that suppression of pro-inflammatory response in replication stage is advantageous to E. coli for the establishment of meningitis [80]. Administration of a single dose of 5 μg of recombinant human IL-10 during bacteremia stages completely cleared the bacteria from the circulation and reversed sustained brain damage within four days post-infection.

**Bacterial translocation into the CNS**

BMEC form the BBB that prevents the transport of harmful substances and pathogenic microorganisms from the blood to the brain. The development of high-grade bacteremia is a prerequisite for E. coli to interact with the BBB. All of the surface structures of E. coli K1 have potential to interact with BMEC for invasion and entry to the CNS. One of the surface appendages in E. coli, S-fimbriae (Sfa) that specifically interacts with NeuAcα2,3Gal1,3GlcNAc epitopes present on glycoproteins is shown to be responsible for binding to BMEC via SfaS adhesin present at the tip of Sfa [129]. However, Sfa plays no significant role in the invasion of HBMEC. Subsequent studies have demonstrated that type-1 fimbriae, which bind to mannose residues of glycoproteins, also contribute to the invasion of E. coli in HBMEC [139]. Nonetheless, when the type-1 fimbriae expression was similar to wild-type E. coli by keeping the fimH operon, which encodes the tip of type-1 fimbriae, in “ON” phase in an OmpA− E. coli, the bacterium could not invade. Furthermore, pretreatment of E. coli with α-methyl mannoside (an inhibitor of type-1 fimbriae) did not show any difference in the invasion, indicating that OmpA is the major determinant in E. coli invasion of HBMEC [63].

OmpA has been shown to bind to BMEC for invasion via a lectin-like activity specific to GlcNAc1, 4GlcNAc (chitobiose) epitopes attached to asparagine-linked glycoproteins [99]. Corroborating the requirement of chitobiose moieties for the pathogenesis, treatment of E. coli with chitooligosaccharides prior to infecting newborn rats prevented the occurrence of meningitis. Subsequent studies have identified a β-form of gp96, a heat-shock protein, present in HBMEC (designated as Ecpg96), which acts as a receptor for OmpA binding to and invasion of the cells. Ecpg96 is an 803 amino acid protein with a weak transmembrane domain [98]. The interaction of OmpA of E. coli with two N-glycosylation sites of Ecpg96 further enhances the expression of the receptor to which additional bacteria bind and invade HBMEC [63]. Additionally, the C-terminal domains of Ecpg96 are required for induction of signaling network to enter HBMEC [76]. E. coli interaction with HBMEC also triggers the expression of TLR2 at the surface, which forms a complex with Ecpg96 while OmpA− E. coli enhanced TLR4 expression and does not associate with the receptor [60]. Consistent with the requirement of TLR2 interaction with Ecpg96 TLR2+/− newborn mice are resistant to infection while TLR4−/− animals are very vulnerable to the development of meningitis.

For internalization, E. coli induces actin cytoskeletal rearrangements to trigger zipper-like mechanism in HBMEC, which engulfs the bacterium into the cell. Besides actin microfilaments, E. coli K1 also requires microtubules for invasion, which probably provides pulling force in HBMEC to internalize the bacteria. E. coli entry induces the phosphorylation of tyrosine residues of focal adhesion kinase (FAK), which is independent of Src kinase activity [105]. PI3-kinase activity is also critical for E. coli invasion of HBMEC, which subsequently activates PLCγ for the influx of extracellular calcium and mobilization of intracellular calcium [104, 130]. This calcium mobilization activates PKC-α, which interacts with caveolin-1, a 22 kDa protein present in caveolae of plasma membranes inducing the ingestion of E. coli by HBMEC [132]. Activated PKC-α associates with VE-cadherin, an adherens junction molecule, and releases β-catenin from the junctions, thereby increasing the permeability of HBMEC monolayers [131]. Pre-incubation of E. coli with anti-OmpA antibodies or HBMEC with anti-Ecpg96 antibodies decreased E. coli-induced permeability confirming that OmpA-Ecpg96 interaction is critical for tight junction disruption.

There is ample evidence that nitric oxide (NO) acts as an antimicrobial molecule and a mediator of cerebral vascular permeability. E. coli upon invasion of HBMEC also produces higher amounts of NO by activating inducible nitric oxide synthase (iNOS) and generating cyclic GMP (cGMP), an important target downstream of NO/IL-10 during bacteremia stages completely cleared the bacterium from the circulation and reversed sustained brain damage within four days post-infection.
hydroxyl pyrimidine (DAHP) pretreatment of HBMEC blocked the invasion in the cells. Both aminoguanidine and DAHP inhibited the onset of meningitis in 3-day-old mice by *E. coli*, highlighting the significance of NO production in the pathogenesis [79, 121]. In addition, screening of a small molecule library using HBMEC invasion assays recognized Telmisartan, an angiotensin II receptor 1 (AT1R) blocker as a potent inhibitor of invasion [64]. Follow-up experiments demonstrated that AT1R forms a complex with Ecgp96 during *E. coli* invasion of HBMEC. Newborn mice pretreated with TS are resistant to both the development of bacteremia and the entry of bacteria into the brain. These experiments clearly demonstrate that targeting Ecgp96 would be beneficial for averting *E. coli*-induced meningitis.

**Immune activation and inflammatory response in the brain**

The survival and multiplication of *E. coli* in PMNs and macrophages result in the production of pro-inflammatory cytokines in the blood, which upregulates the expression of intracellular adhesion molecule 1 (ICAM-1) on the BBB. In addition, the interaction of OmpA of *E. coli* with Ecgp96 on HBMEC induces ICAM-1 expression, thereby enhancing the binding of THP-1 cells in culture [117]. This upregulation of ICAM-1 expression aids in the infiltration of PMNs during the onset of meningitis. Furthermore, gliosis and neuronal apoptosis in both cortex and hippocampus and the production of greater amounts of TNF-α and IL1β have been observed in the brains of newborn mice infected with *E. coli* [80]. Nonetheless, the interaction of *E. coli* with neuronal cells and glial cells is poorly studied. Further studies are clearly needed to gain a better understanding of whether the bacteria directly damages the brain or the damage is a causal effect of pro-inflammatory response.

**Conclusions and outlook**

In summary, despite advances in antimicrobial therapy and vaccine development, bacterial meningitis represents a significant cause of morbidity and mortality, mainly in infants, children and in the elderly or immunocompromised patient. The emergence of antibiotic-resistant strains, e.g., *E. coli* and *S. pneumoniae*, phenotypic heterogeneity, e.g., meningococci, the lack of effective vaccines, e.g., GBS, or the occurrence of new emerging diseases as a results of zoonotic species jumps, e.g., *S. suis*, demands alternative strategies to prevent as many cases of bacterial meningitis and the associated neurological sequelae as possible. Significant progress has been made in identifying molecular mechanisms that contribute to host–pathogen interactions during the progression of CNS disease. Identification of common pathways employed by bacterial pathogens to breach mucosal barriers, survive in the blood stream and cross the BBB or B-CSF barrier will assist in the identification of important bacterial and host cell targets for the development of effective therapies. The identification of molecular patterns used by several bacterial species to cross the B-CSFB and BBB may lead to the systemic application of antibodies or antagonists blocking barrier epitopes involved in the attachment and transcytosis of bacteria. Vaccination against these bacterial patterns or prophylactic application of an antagonistic drug with low side effects can be an option, particularly in persons at a high risk of acquiring meningitis. Thus, targeting bacterial components and their associated signaling events should offer novel therapeutic strategies. A multi-disciplinary 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 and control strategies.

**Acknowledgments** Authors are listed in alphabetical order and contributed to this review by providing following chapters: Abstract and Introduction (all authors), *S. pneumoniae* meningitis (E.I. Tuomanen, N. Gratz), *Group B Streptococcus* meningitis (K.S. Doran, B.J. Kim), *Meningococcal* meningitis (A. Schubert-Unkmeir), *S. suis* meningitis (M. Fulde, P. Valentin-Weigand), *E. coli K1* meningitis (N.V. Prasadaro), Common steps and mechanisms in pathogenesis of bacterial meningitis (M. Fulde, P. Valentin-Weigand) and Conclusions and Outlook (R. Nau, P. Valentin-Weigand). Figure 1 was provided by R. Nau. All other figures were provided by respective chapter authors. Elizabeth Rossi is gratefully acknowledged for artwork in Fig. 3. This study was in part supported by Niedersachsen-Research Network on Neuroinfectiology (N-RENT) of the Ministry of Science and Culture of Lower Saxony, Germany, to M. Fulde and P. Valentin-Weigand. The work on pneumococcal meningitis in E.I. Tuomanen’s laboratory is supported by funding from NIH/NIAD R01 27913 and ALSAC. The work presented here from Dr. Prasadaro’s lab has been supported by the grants from the National Institutes of Health, USA (AI40567, NS73115, and HD41525), American Heart Association and Children’s Hospital Los Angeles Career Development Fellowships. Work on the BBB and GBS meningitis in K.S. Doran’s laboratory is supported by funding from NIH/NINDS (Grant No. RO1NS051247). M. Fulde received support by the Freie Universität Berlin within the Excellence Initiative of the German Research Foundation. The work on neuronal injury in Dr. Nau’s lab has been supported by Sparkasse Göttingen. The authors acknowledge their laboratory members and researchers whose work has not been discussed here in detail due to space constraints.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
References

1. Al-Numani D, Segura M, Dore M, Gottschalk M (2003) Up-regulation of ICAM-1, CD11a/CD18 and CD11c/CD18 on human THP-1 monocytes stimulated by Streptococcus suis serotype 2. Clin Exp Immunol 133:67–77.

2. Alkuwaity K, Taylor A, Heckels JE, Doran KS, Christodoulides M (2012) Group B Streptococcus interactions with human meningeal cells and astrocytes in vitro. PLoS One 7:e42660. doi:10.1371/journal.pone.0042660

3. Baker CJ, Edwards MS (2001) Group B streptococcal infection. In: Remington JS, Klein JO (eds) Infectious diseases of the fetus and newborn infant, 5th edn. WB Saunders, Philadelphia, pp 1091–1156

4. Banerjee A, Kim BJ, Carmona EM, Cutting AS, Gurney MA, Carlos C, Feuer R, Prasadarao NV, Doran KS (2011) Bacterial Pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration. Nat Commun 2:462. doi:10.1038/ncomms1474

5. Barichello T, Collodel A, Generoso JS, Simoes LR, Moreira AP, Ceretta RA, Petronilho F, Quevedo J (2015) Targets for adjunctive therapy in pneumococcal meningitis. J Neuroimmunol 278:262–270. doi:10.1016/j.jneuroim.2014.11.015

6. Baums CG, Valentijn-Weigand P (2009) Surface-associated and secreted factors of Streptococcus suis in epidemiology, pathogenesis and vaccine development. Anim Health Res Rev/Conf Res Work Anim Dis 10:65–83. doi:10.1017/S146625230900003X

7. Benga L, Friedl P, Valentijn-Weigand P (2005) Adherence of Streptococcus suis to porcine endothelial cells. J Vet Med B Infect Dis Vet Public Health 52:392–395. doi:10.1111/j.1439-0450.2005.00880.x

8. Benga L, Goethe R, Rohde M, Valentijn-Weigand P (2004) Non-encapsulated strains reveal novel insights in invasion and survival of Streptococcus suis in epithelial cells. Cell Microbiol 6:867–881. doi:10.1111/j.1462-5822.2004.00409.x

9. Bergmann S, Lang A, Rohde M, Agarwal V, Rennemeier C, Grashoff C, Preissner KT, Hammerschmidt S (2009) Integrin-linked kinase is required for vitronectin-mediated internalization of Streptococcus suis by host cells. J Cell Sci 122:256–267. doi:10.1242/jcs.035600

10. Berman PH, Banker BQ (1966) Neonatal meningitis—a clinical and pathological study of 29 cases. Pediatrics 38:6–24

11. Bernard SC, Simpson N, Join-Lambert O, Federici C, Laran-Chich MP, Maissa N, Bouzinba-Segard H, Morand PC, Chretien F, Taouji S, Chevet E, Janel S, Lafont F, Coureuil M, Segura A, Niedergang F, Marullo S, Couraud PO, Nassif X, Bourdoulous S (2014) Pathogenic Neisseria meningitidis utilizes CD147 for vascular colonization. Nat Med 20:725–731. doi:10.1038/nm.3563

12. Bradley CJ, Griffiths NJ, Rowe HA, Heyderman RS, Virji M (2005) Critical determinants of the interactions of capsule-expressing Neisseria meningitidis with host cells: the role of receptor density in increased cellular targeting via the outer membrane Opa proteins. Cell Microbiol 7:1490–1503. doi:10.1111/j.1462-5822.2005.00572.x

13. Brandt CT, Lundgren JD, Land SP, Frimodt-Moller N, Christophersen T, Benfield T, Espersen F, Hougaard DM, Ostergaard C (2004) Attenuation of the bacterial load in blood by pre-treatment with granulocyte-colony-stimulating factor protects rats from fatal outcome and brain damage during Streptococcus pneumoniae meningitis. Infect Immun 72:4647–4653. doi:10.1128/IAI.72.9.4647-4653.2004

14. Braun JS, Novak R, Herzog KH, Bodner SM, Cleveland JL, Tuomanen EI (1999) Neuroprotection by a caspase inhibitor in acute bacterial meningitis. Nat Med 5:298–302. doi:10.1038/6514

15. Chang YC, Wang Z, Flax LA, Xu D, Esko JD, Nizet V, Baron MJ (2011) Glycans on pneumococci facilitate entry of a bacterial pathogen into central nervous systems. PLoS Pathog 7:e1002082. doi:10.1371/journal.ppat.1002082

16. Charland N, Nizet V, Rubens CE, Kim KS, Lacouture S, Gottschalk M (2000) Streptococcus suis serotype 2 interactions with human brain microvascular endothelial cells. Infect Immun 68:637–643

17. Coureuil M, Lecuyer H, Scott MG, Boularan C, Enslen H, Soyer M, Mikaty G, Bourdoulous S, Nassif X, Marullo S (2010) Meningococcus Hijacks a beta2-adrenoceptor/beta-Arrestin pathway to cross brain microvasculature endothelium. Cell 143:1149–1160. doi:10.1016/j.cell.2010.11.035

18. Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S, Dumenil G, Mege RM, Wekslers BB, Romero IA, Couraud PO, Nassif X (2009) Meningococcal type IV pilin recruit the polarity complex to cross the brain endothelium. Science 325:83–87. doi:10.1126/science.1173196

19. Coutinho LG, Grandgirard D, Leib SL, Agnez-Lima LF (2013) Cerebrospinal-fluid cytokine and chemokine profile in patients with pneumococcal and meningococcal meningitis. BMC Infect Dis 13:326. doi:10.1186/1471-2334-13-326

20. Cumley NJ, Smith LM, Anthony M, May RC (2012) The CovS/CovR acid response regulator is required for intracellular survival of group B Streptococcus in macrophages. Infect Immun 80:1650–1661. doi:10.1128/IAI.05443-11

21. Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I. Tuomanen EI (1995) Streptococcus pneumoniae anchor to activated human cells by the receptor for platelet-activating factor. Nature 377:435–438. doi:10.1038/377435a0

22. Cundell DR, Weiser JN, Shen J, Young A, Tuomanen EI (1995) Relationship between colonial morphology and adherence of Streptococcus pneumoniae. Infect Immun 63:757–761

23. Cutting AS, Del Rosario Y, Mu R, Rodriguez A, Till A, Subramani S, Gottlieb RA, Doran KS (2014) The role of autophagy during group B Streptococcus infection of blood-brain barrier endothelium. J Biol Chem 289:35711–35723. doi:10.1074/jbc.M114.588657

24. Dacey RG, Sande MA (1974) Effect of probenecid on cerebrospinal fluid concentrations of penicillin and cephalosporin derivatives. Antimicrob Agents Chemother 6:437–441

25. de Buhr N, Neumann A, Jerjomicheva N, von Kockritz-Blickwede M, Baums CG (2014) Streptococcus suis DNase SsnA contributes to degradation of neutrophil extracellular traps (NETs) and evasion of NET-mediated antimicrobial activity. Microbiology 160:385–395. doi:10.1099/mic.0.072199-0

26. de Buhr N, Stehr M, Neumann A, Naim HY, Valentijn-Weigand P, von Kockritz-Blickwede M, Baums CG (2015) Identification of a novel DNase of Streptococcus suis (EndAsuis) important for neutrophil extracellular trap degradation during exponential growth. Microbiology 161:838–850. doi:10.1099/mic.0.000040

27. Dominguez-Punaro MC, Segura M, Plante MM, Lacouture S, Rivest S, Gottschalk M (2007) Streptococcus suis serotype 2, an important swine and human pathogen, induces strong systemic and cerebral inflammatory responses in a mouse model of infection. J Immunol 179:1842–1854

28. Dominguez-Punaro Mde L, Segura M, Contreras I, Lachance C, Houde M, Lecours MP, Olivier M, Gottschalk M (2010) In vitro characterization of the microglial inflammatory response to Streptococcus suis, an important emerging zoonotic agent of meningitis. Infect Immun 78:5074–5085. doi:10.1128/IAI.00698-10

29. Doran KS, Engelson EJ, Khoosavi A, Maisey HC, Fedtke I, Equils O, Michelsen KS, Arditii M, Peschel A, Nizet V (2005)
Blood-brain barrier invasion by group B Streptococcus depends upon proper cell-surface anchoring of lipoteichoic acid. J Clin Invest 115:2499–2507. doi:10.1172/JCI23829

30. Doran KS, Liu GY, Nizet V (2003) Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J Clin Investig 112:736–744. doi:10.1172/JCI17335

31. Edmond KM, Kortalsouadaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, Heath PT (2012) Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet 379:547–556. doi:10.1016/S0140-6736(11)61651-6

32. Edwards MS, Rench MA, Haffar AA, Murphy MA, Desmond MM, Baker CJ (1985) Long-term sequelae of group B streptococcal meningitis in infants. J Pediatr 106:717–722

33. Eugene E, Hoffmann I, Pujol C, Couraud PO, Bourdoulous S, Fagan RP, Lambert MA, Smith SG (2008) The hek outer membrane protein of \textit{Escherichia coli} strain RS218 binds to proteoglycan and utilizes a single extracellular loop for adhesion, invasion, and autoggregation. Infect Immun 76:1135–1142. doi:10.1128/IAI.01327-07

34. Fagan RP, Lambert MA, Smith SG (2008) The hek outer membrane protein of \textit{Escherichia coli} mediates adhesion to porcine epithelium and mucus. Microbiology 154:2818–2828. doi:10.1099/mic.0.037960-0

35. Fillon S, Soulis K, Rajasekaran S, Benedict-Hamilton H, Radin JN, Orihuela CJ, El Kasmi KC, Murti G, Kaushal D, Gaber MW, Weber JR, Murray PJ, Tuomanen EI (2006) Platelet-activating factor receptor for pathogenic \textit{Neisseria meningitidis} and is independent of Toll-like receptor (TLR)4 and TLR2 signalling. Cell Microbiol 7:415–430. doi:10.1111/j.1462-5822.2004.00471.x

36. Fittipaldi N, Sekizaki T, Takahashi S, Oliver KA, Chan MS, Kunst F, Glaser P, Rusiniok C, Crook DW, Harding RM, Bisharant N, Spratt BG (2003) Multilocus sequence typing system for group B streptococcus. J Clin Microbiol 41:2530–2536

37. Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Dominguez-Munoz M, Willenborg J, de Greeff A, Bacterium suis. Future Microbiol 7:3587–3594. doi:10.1128/IAI.01568-07

38. Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Dominguez-Munoz M, Wehe M, Weber JR, Murray PJ, Tuomanen EI (2006) Platelet-activating factor receptor and innate immunity: uptake of gram-positive bacterial cell wall into host cells and cell-specific pathophysiology. J Immunol 177:6182–6191

39. Fittipaldi N, Segura M, Grenier D, Gottschalk M (2012) Virulence factors involved in the pathogenesis of the infection caused by the swine pathogen and zoonotic agent \textit{Streptococcus suis}. Future Microbiol 7:259–279. doi:10.2217/fmb.11.149

40. Fittipaldi N, Sekizaki T, Takamatsu D, de la Cruz Dominguez-Munoz M, Willenborg J, de Greeff A, Bacterium suis. Future Microbiol 7:3587–3594. doi:10.1128/IAI.01568-07

41. Fittipaldi N, Sekizaki T, Takamatsu D, Harel J, Dominguez-Munoz M, Wehe M, Weber JR, Murray PJ, Tuomanen EI (2006) Platelet-activating factor receptor and innate immunity: uptake of gram-positive bacterial cell wall into host cells and cell-specific pathophysiology. J Immunol 177:6182–6191

42. Gottschalk M, Xu J, Calzas C, Segura M (2010) \textit{Streptococcus suis}: a new emerging or an old neglected zoonotic pathogen? Future Microbiol 5:371–391. doi:10.2217/fmb.10.2

43. Gould JM, Weiser JW (2002) The inhibitory effect of C-reactive protein on bacterial phosphorylcholine platelet-activating factor receptor-mediated adhesion is blocked by surfactant. J Infect Dis 186:361–371. doi:10.1086/341658

44. Grassme H, Becker KA (2013) Bacterial infections and cereamide. Handbook of experimental pharmacology.
58. Koppe U, Sutorp N, Optiz B (2012) Recognition of Streptococcus pneumoniae by the innate immune system. Cell Microbiol 14:460–466. doi:10.1111/j.1462-5822.2011.01746.x
59. Kostyukova NN, Volkova MO, Ivanova VV, Kvetnaya AS (1995) A study of pathogenic factors of Streptococcus pneumoniae strains causing meningitis. FEMS Immunol Med Microbiol 10:133–137
60. Krishnan S, Chen S, Turcatel G, Arditi M, Prasadarao NV
61. Krishnan S, Liu F, Abrol R, Hodges J, Goddard WA, Prasadarao NV (2012) Outer membrane protein A for entry into macrophages experimental and computational analysis. J Biol Chem 289:30937–30949. doi:10.1074/jbc.M111.599407
62. Krishnan S, Prasadarao NV (2012) Outer membrane protein A and OprF: versatile roles in Gram-negative bacterial infections. FEBS J 279:919–931. doi:10.1111/j.1742-4658.2012.08482.x
63. Krishnan S, Prasadarao NV (2014) Identification of minimum carbohydrate moiety in N-glycosylation sites of brain endothelial cell glycoprotein K1 for interaction with Escherichia coli K1 outer membrane protein A. Microbes Infect/Inst Pasteur 16:540–552. doi:10.1016/j.micinf.2014.06.002
64. Krishnan S, Shanmuganathan MV, Behenna D, Stoltz BM, Leib SL, Heimgartner C, Bifrare YD, Loeffler JM, Taauber (2011) Pathogenesis and pathophysiology of pneumococcal meningitis in infant rats: role of bacteremia in pathogenesis of age-dependent inflammatory responses in cerebrospinal fluid. J Infect Dis 207:1307–1319. doi:10.1086/jem.20092265
65. Mittal R, Prasadarao NV (2011) gp96 expression in neutrophils is critical for the onset of Escherichia coli K1 (RS218) meningitis. Nature Commun 2:552. doi:10.1038/Ncomms1554 (Art552)
66. Mook-Kanamori BB, Geldhoff M, van der Poll T, van de Beek D (2011) Pathogenesis and pathophysiology of pneumococcal meningitis. Clin Microbiol Rev 24:557–591. doi:10.1128/CMR.00008-11
67. Moxon ER, Ostrow PT (1977) Haemophilus influenzae meningitis in infant rats: role of bacteremia in pathogenesis of age-dependent inflammatory responses in cerebrospinal fluid. J Infect Dis 135:303–307
68. Mu R, Kim BJ, Paco C, Del Rosario Y, Courtney HS, Doran KS (2014) Identification of a group B streptococcal fibronectin binding protein, SfBA, that contributes to invasion of brain endothelium and development of meningitis. Infect Immun 82:2276–2286. doi:10.1128/IAI.01559-13
69. Nau R, Bruck W (2002) Neuronal injury in bacterial meningitis: mechanisms and implications for therapy. Trends Neurosci 25:38–45
70. Nau R, Gerber J, Bunkowski S, Bruck W (2004) Axonal injury, a neglected cause of CNS damage in bacterial meningitis. Neurology 62:509–511
71. Nau R, Soto A, Bruck W (1999) Apoptosis of neurons in the dentate gyrus in humans suffering from bacterial meningitis. J Neuropathol Exp Neurol 58:265–274
72. Nealon TJ, Mattingly SJ (1983) Association of elevated levels of cellular lipoteichoic acids of group B streptococci with human neonatal disease. Infect Immun 39:1243–1251
90. Nizet V, Kim KS, Stins M, Jonas M, Chi EY, Nguyen D, Rubens CE (1997) Invasion of brain microvascular endothelial cells by group B streptococci. Infect Immun 65:5074–5081

91. Ohrihuela CJ, Mahdavi J, Thornton J, Mann B, Wooldridge KG, Abouseada N, Oldfield NJ, Self T, Ala’Aldeen DA, Tuomanen EI (2009) Laminin receptor initiates bacterial contact with the blood brain barrier in experimental meningitis models. J Clin Invest 119:1638–1646. doi:10.1172/JCI36759

92. Ostergaard C, Benfield T, Gesser B, Kharazmi A, Frimodt-Moller N, Ebers S, Lundgren JD (1999) Pretreatment with granulocyte colony-stimulating factor attenuates the inflammatory response but not the bacterial load in cerebrospinal fluid during experimental pneumococcal meningitis in rabbits. Infect Immun 67:3430–3436

93. Patterson H, Saralabhi A, Parikka M, Dramsi S, Trieu-Cuot P, Paul R, Koedel U, Pfister HW (2003) Using knockout mice to study experimental meningitis. Arch Immunol Ther Exp 51:315–326

94. Petersdorf RG, Swarner DR, Garcia M (1962) Studies on the pathogenesis of meningitis. II. Development of meningitis during pneumococcal bacteremia. J Clin Invest 41:320–327. doi:10.1172/JCI104485

95. Prasadarao NV, Blom AM, Villoutreix BO, Linsangan LC, Reddy MA, Wass CA, Kim KS, Schlaepfer DD, Prasadarao NV (2005) Involvement of focal adhesion kinase in K1 invasion of human brain Microvascular endothelial cells. Infect Immun 73:7827–7835. doi:10.1128/IAI.73.4.7827-7835.2005

96. Petersdorf RG, Swarner DR, Garcia M (1962) Studies on the pathogenesis of meningitis. II. Development of meningitis during pneumococcal bacteremia. J Clin Invest 41:320–327. doi:10.1172/JCI104485

97. Prasadarao NV, Srivastava PK, Rudrabhatla RS, Kim KS, Paul R, Koedel U, Pfister HW (2002) A novel interaction of outer membrane protein A with C4b binding protein mediates serum resistance of Escherichia coli K1. J Immunol 169:6352–6360

98. Prasadarao NV, Srivastava PK, Rudrabhatla RS, Kim KS, Paul R, Koedel U, Pfister HW (2002) A novel interaction of outer membrane protein A with C4b binding protein mediates serum resistance of Escherichia coli K1. J Immunol 169:6352–6360

99. Prasadarao NV, Wass CA, Kim KS (1996) Endothelial cell GlcNAc beta 1-4GlcNAc epitopes for outer membrane protein A enhance traversal of Escherichia coli across the blood-brain barrier. Infect Immun 64:154–160

100. Pron B, Taha MK, Rambaud C, Fournet JC, Pattey N, Monnet JP, Musilek M, Beretti JL, Nassif X (1997) Interaction of Neisseria meningitidis with the components of the blood-brain barrier correlates with increased expression of PIIC. J Infect Dis 176:1285–1292

101. Quach D, van Sorge NM, Kristian SA, Bryan JD, Shelver DW, Doran KS (2009) The CiaR response regulator in group B Streptococcus promotes intracellular survival and resistance to innate immune defenses. J Bacteriol 191:2023–2032. doi:10.1128/JB.00126-08

102. Quirante J, Ceballos R, Cassidy G (1974) Group B beta-hemolytic streptococcal infection in the newborn. I. Early onset infection. Am J Dis Child 128:659–665

103. Radin JN, Ohrihuela CJ, Murti G, Guglielmo CI, Murray PJ, Tuomanen EI (2005) Beta-arrestin 1 participates in platelet-activating factor receptor-mediated endocytosis of Streptococcus pneumoniae. Infect Immun 73:7827–7835. doi:10.1128/IAI.73.12.7827-7835.2005

104. Reddy MA, Prasadarao NV, Wass CA, Kim KS (2000) Phosphatidylinositol 3-kinase activation and interaction with focal adhesion kinase in Escherichia coli K1 invasion of human brain microvascular endothelial cells. J Biol Chem 275:36769–36774. doi:10.1074/jbc.M007382200

105. Reddy MA, Wass CA, Kim KS, Schlæpfer DD, Prasadarao NV (2000) Involvement of focal adhesion kinase in Escherichia coli invasion of human brain microvascular endothelial cells. Infect Immun 68:6423–6430

106. Reiss A, Braun JS, Jager K, Frey D, Laube G, Buhrer C, Felderhoff-Müser U, Stadelmann C, Nizet V, Weber JR (2011) Bacterial pore-forming cytolsins induce neuronal damage in a rat model of neonatal meningitis. J Infect Dis 203:393–400. doi:10.1093/infdis/jiq047

107. Ring A, Weiser JN, Tuomanen EI (1998) Pneumococcal trafficking across the blood-brain barrier. Molecular analysis of a novel bidirectional pathway. J Clin Invest 102:347–360. doi:10.1172/JCI2406

108. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM (2001) Meningococcal disease. N Engl J Med 344:1378–1388. doi:10.1056/NEJM200010053341807

109. Rubens CE, Wessels MR, Heggen LM, Kasper DL (1987) Transposon mutagenesis of type III group B Streptococcus: correlation of capsule expression with virulence. Proc Natl Acad Sci USA 84:7208–7212

110. Sa ECC, Griffiths NJ, Virji M (2010) Neisseria meningitidis Opc invasin binds to the sphingolipid tyrosines of activated vitronectin to attach to and invade human brain endothelial cells. PLoS Pathog 6:e1000991. doi:10.1371/journal.ppat.1000991

111. Sadarangani M, Pollard AJ, Gray-Owen SD (2011) Opa proteins and CEACAMs: pathways of immune engagement for pathogenic Neisseria. FEMS Microbiol Rev 35:498–514. doi:10.1111/j.1574-6976.2010.00260.x

112. Sarkari J, Pandit N, Moxon ER, Achtman M (1994) Variable expression of the Opc outer membrane protein in Neisseria meningitidis is caused by size variation of a promoter containing poly-cytidine. Mol Microbiol 13:207–217

113. Scheld WM, Koedel U, Nathan B, Pfister HW (2002) Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. J Infect Dis 186(Suppl 2):S225–S233. doi:10.1086/344939

114. Schubert-Unkmeir A, Konrad C, Slanina H, Czapek F, Hebling S, Frosch M (2010) Neisseria meningitidis induces brain microvascular endothelial cell detachment from the matrix and cleavage of occludin: a role for MMP-8. PLoS Pathog 6:e1000874. doi:10.1371/journal.ppat.1000874

115. Seele J, Beineke A, Hillermann LM, Jaschok-Kentner B, von Pawel-Rammingen U, Valentin-Weigand P, Baums CG (2015) The immunoglobulin M-degrading enzyme of Streptococcus suis, IdeSsuis, is involved in complement evasion. Vet Res 46:45. doi:10.1186/s13567-015-0171-6

116. Seitz M, Baums CG, Neis C, Benga L, Fulde M, Rohde M, Goethe R, Valentin-Weigand P (2013) Subcytolytic effects of suilysin on interaction of Streptococcus suis with epithelial cells. Vet Microbiol 167:584–591. doi:10.1086/673859

117. Sellaradj SK, Periandrythevar P, Prasadarao NV (2007) Outer membrane protein A of Escherichia coli K1 selectively enhances the expression of intercellular adhesion molecule-1 in brain microvascular endothelial cells. Microbes Infect/Inst Pasteur 9:547–557. doi:10.1016/j.micinf.2007.01.020

118. Sellaradj SK, Prasadarao NV (2005) Escherichia coli K1 inhibits its proinflammatory cytokine induction in monocytes by preventing NF-kappaB activation. J Leukoc Biol 78:544–554. doi:10.1189/jlb.0904516

119. Seo HS, Minasov G, Seepersaud R, Doran KS, Dubrovskova I, Shuvalova L, Anderson WF, Iverson TM, Sullam PM (2013) Characterization of fibrinogen binding by glycoproteins Srr1 and Srr2 of Streptococcus agalactiae. J Biol Chem 288:35982–35996. doi:10.1074/jbc.M113.513358

120. Seo HS, Mu R, Kim BJ, Doran KS, Sullam PM (2012) Binding of glycoprotein Srr1 of Streptococcus agalactiae to fibrinogen promotes attachment to brain endothelium and the development of
of meningitis. PLoS Pathog 8:e1002947. doi:10.1371/journal.ppat.1002947

Shanmuganathan MV, Krishnan S, Fu X, Prasadarao NV (2013) Attenuation of biotin synthase prevents Escherichia coli K1 invasion of brain endothelial cells and the development of meningitis in newborn mice. J Infect Dis 207:61–71. doi:10.1093/infdis/jis565

Shanmuganathan MV, Krishnan S, Fu X, Prasadarao NV (2014) Escherichia coli K1 induces pertin idurion for enhanced expression of Fc gamma receptor I to invade RAW 264.7 macrophages. Microbes Infect/Inst Pasteur 16:134–141. doi:10.1016/j.micinf.2013.10.013

Simonis A, Hebling S, Guibins E, Schneider-Schaullies S, Schubert-Unkmeir A (2012) Role of epidermal growth factor receptor signaling in the interaction of Neisseria meningitidis with endothelial cells. Infect Immun 80:1243–1255. doi:10.1128/IAI.01346-13

Smith SG, Mahon V, Lambert MA, Fagan RP (2007) A molecular Swiss army knife: OmpA structure, function and expression. FEMS Microbiol Lett 273:1–11. doi:10.1111/j.1574-6968.2007.00778.x

Sokolova O, Heppel N, Jagerhuber R, Kim KS, Frosch M, Eigenthaler M, Schubert-Unkmeir A (2004) Interaction of Neisseria meningitidis with human brain microvascular endothelial cells: role of MAP- and tyrosine kinases in invasion and inflammatory cytokine release. Cell Microbiol 6:1153–1166. doi:10.1111/j.1462-5822.2004.00422.x

Stins MF, Prasadarao NV, Ibric L, Wass CA, Luckett P, Kim KS (1994) Binding characteristics of S fimbriated Escherichia coli to isolated brain microvascular endothelial cells. Am J Pathol 145:1228–1236

Sukumaran SK, McNamara G, Prasadarao NV (2003) Escherichia coli K1 invasion increases human brain microvascular endothelial cell monolayer permeability by disassembling vascular-endothelial cadherins at tight junctions. J Infect Dis 188:1295–1309. doi:10.1086/379042

Sukumaran SK, Quon MJ, Prasadarao NV (2002) Escherichia coli K1 internalization via caveolae requires caveolin-1 and protein kinase Calpha interaction in human brain microvascular endothelial cells. J Biol Chem 277:50716–50724. doi:10.1074/jbc.M208830200

Sukumaran SK, Selvaraj SK, Prasadarao NV (2004) Inhibition of apoptosis by Escherichia coli K1 is accompanied by increased expression of BCLXL and blockade of mitochondrial cytochrome c release in macrophages. Infect Immun 72:6012–6022. doi:10.1128/IAI.72.10.6012-6022.2004

Sukumaran SK, Shimada H, Prasadarao NV (2003) Entry and intracellular replication of Escherichia coli K1 in macrophages require expression of outer membrane protein A. Infect Immun 71:5951–5961

Tauber MG, Kennedy SL, Tureen JH, Lowenstein DH (1992) Experimental pneumococcal meningitis causes central nervous system pathology without inducing the 72-kd heat shock protein. Am J Pathol 141:53–60

Tazi A, Disson O, Bellais S, Bouabaud A, Dmytruk N, Dramsi S, Mistou MY, Khun H, Meclier C, Tardeix I, Trieu-Cuot P, Lecuit M, Poyart C (2010) The surface protein HvgA mediates group B streptococcus hypervirulence and meningococcal tropism in neonates. J Exp Med 207:2313–2322. doi:10.1084/jem.20092594

Tenenbaum T, Matalon D, Adam R, Seibt A, Wewer C, Schwerk C, Gallus HJ, Schroten H (2008) Dexamethasone prevents alteration of tight junction-associated proteins and barrier function in porcine choroid plexus epithelial cells after infection with Streptococcus suis in vitro. Brain Res 1229:1–17. doi:10.1016/j.brainres.2008.06.118

Tenenbaum T, Papandreu T, Gellrich D, Friedrichs U, Seibt A, Adam R, Wewer C, Gallus HJ, Schwerk C, Schroten H (2009) Polar bacterial invasion and translocation of Streptococcus suis across the blood-cerebrospinal fluid barrier in vitro. Cell Microbiol 11:323–336. doi:10.1111/j.1462-5822.2008.01255.x

Teng CH, Cai M, Shin S, Xie Y, Kim KJ, Khan NA, Di Cello F, Kim KS (2005) Escherichia coli K1 RS218 interacts with human brain microvascular endothelial cells via type 1 fimbria bacteria in the fimbriated state. Infect Immun 73:2923–2931. doi:10.1128/IAI.73.5.2923-2931.2005

Tibousse D, Sinclair A, Yau I, Teatero S, Fittipaldi N, Richardson SE, Mayatepek E, Jahn P, Askalan R (2015) Late-onset group B streptococcal meningitis has cerebrovascular complications. J Pediatr 166(1187–1192):e1181. doi:10.1016/j.jpeds.2015.02.014

Tuomanen E, Liu H, Hengstler B, Zak O, Tomasz A (1985) The induction of meningial inflammation by components of the pneumococcal cell wall. J Infect Dis 151:859–868

Uchiyama S, Carlin AF, Weiman S, Banerjee A, Quach D, Hightower G, Mitchell TJ, Doran KS, Nizet V (2009) The surface-anchored NanA protein promotes pneumococcal brain endothelial cell invasion. J Exp Med 206:1845–1852. doi:10.1084/jem.20090386

Unkmeir A, Latsch K, Dietrich G, Wintermeyer E, Schinke B, Schwender S, Kim KS, Eigenthaler M, Frosch M (2002) Fibronectin mediates Op8-dependent internalization of Neisseria meningitidis in human brain microvascular endothelial cells. Mol Microbiol 46:933–946

Vadeboncoeur N, Segura M, Al-Numani D, Vanier G, Adam R, Wewer C, Gallus HJ, Schwerk C, Schroten H (2010) Pneumococcal carriage results in ganglioside-mediated olfactory tissue infection. Proc Natl Acad Sci USA 107:10365–10370. doi:10.1073/pnas.1003900107

Van Ginkel FW, McGhee JR, Watt JM, Campos-Torres A, Parish LA, Briles DE (2003) Pneumococcal carriage results in ganglioside-mediated olfactory tissue infection. Proc Natl Acad Sci USA 100:14363–14367. doi:10.1073/pnas.2235844100

Van Putten JP, Paul SM (1995) Binding of syndecan-like cell surface proteoglycan receptors is required for Neisseria gonorrhoeae entry into human mucosal cells. The EMBO J 14:2144–2154

Virji M (2009) Pathogenic neisseriae: surface modulation, pathogenesis and infection control. Nat Rev Microbiol 7:274–286. doi:10.1038/nrmicro2097
149. Waage A, Brandtzæg P, Halstensen A, Kierulf P, Espevik T (1989) The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 169:333–338

150. Watt JP, Wolfson LJ, O’Brien KL, Henkle E, Deloria-Knoll M, McCall N, Lee E, Levine OS, Hajjeh R, Mulholland K, Cherian T, Hib, Pneumococcal Global Burden of Disease Study T (2009) Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. Lancet 374:903–911. doi:10.1016/S0140-6736(09)61203-4

151. Wertheim HF, Nghia HD, Taylor W, Schultz C (2009) *Streptococcus suis*: an emerging human pathogen. Clin Infect Dis Off Publ Infect Dis Soc Am 48:617–625. doi:10.1086/596763

152. Wewer C, Seibt A, Wolburg H, Greune L, Schmidt MA, Berger J, Galla HJ, Quitsch U, Schwerk C, Schloten H, Tenenbaum T (2011) Transcellular migration of neutrophil granulocytes through the blood-cerebrospinal fluid barrier after infection with *Streptococcus suis*. J Neuroinflamm 8:51. doi:10.1186/1742-2094-8-51

153. Whalen CM, Hockin JC, Ryan A, Ashton F (1995) The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992. Emergence of a virulent clone of *Neisseria meningitidis*. JAMA 273:390–394

154. Willenborg J, Fulde M, de Greeff A, Rohde M, Smith HE, Valentín-Weigand P, Goethe R (2011) Role of glucose and CcpA in capsule expression and virulence of *Streptococcus suis*. Microbiology 157:1823–1833. doi:10.1099/mic.0.046417-0

155. Wippel C, Maurer J, Fortsch C, Hupp S, Bohl A, Ma J, Mitchell TJ, Bunkowski S, Bruck W, Nau R, Iliev AI (2013) Bacterial cytolsin during meningitis disrupts the regulation of glutamate in the brain, leading to synaptic damage. PLoS Pathog 9:e1003380. doi:10.1371/journal.ppat.1003380

156. Wooster DG, Maruvada R, Blom AM, Prasadaro NV (2006) Logarithmic phase *Escherichia coli* K1 efficiently avoids serum killing by promoting C4 bp-mediated C3b and C4b degradation. Immunology 117:482–493. doi:10.1111/j.1365-2567.2006.02323.x

157. Wu Z, Wu C, Shao J, Zhu Z, Wang W, Zhang W, Tang M, Pei N, Fan H, Li J, Yao H, Gu H, Xu X, Lu C (2014) The *Streptococcus suis* transcriptional landscape reveals adaptation mechanisms in pig blood and cerebrospinal fluid. RNA 20:882–898. doi:10.1261/rna.041822.113

158. Zhang A, Mu X, Chen B, Liu C, Han L, Chen H, Jin M (2010) Identification and characterization of IgA1 protease from *Streptococcus suis*. Vet Microbiol 140:171–175. doi:10.1016/j.vetmic.2009.06.034

159. Zhang JR, Mostov KE, Lamm ME, Nanno M, Shimida S, Ohwaki M, Tuomanen E (2000) The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell 102:827–837

160. Zheng H, Sun H, Dominguez-Punaro Mde L, Bai X, Ji S, Segura M, Xu J (2013) Evaluation of the pathogenesis of meningitis caused by *Streptococcus suis* sequence type 7 using the infection of BV2 microglial cells. J Med Microbiol 62:360–368. doi:10.1099/jmm.0.046698-0

161. Zysk G, Bruck W, Gerber J, Bruck Y, Prange HW, Nau R (1996) Anti-inflammatory treatment influences neuronal apoptotic cell death in the dentate gyrus in experimental pneumococcal meningitis. J Neuropathol Exp Neurol 55:722–728

162. Zysk G, Schneider-Wald BK, Hwang JH, Bejo L, Kim KS, Mitchell TJ, Hakenbeck R, Heinz HP (2001) Pneumolysin is the main inducer of cytotoxicity to brain microvascular endothelial cells caused by *Streptococcus pneumoniae*. Infect Immun 69:845–852. doi:10.1128/iai.69.2.845-852.2001